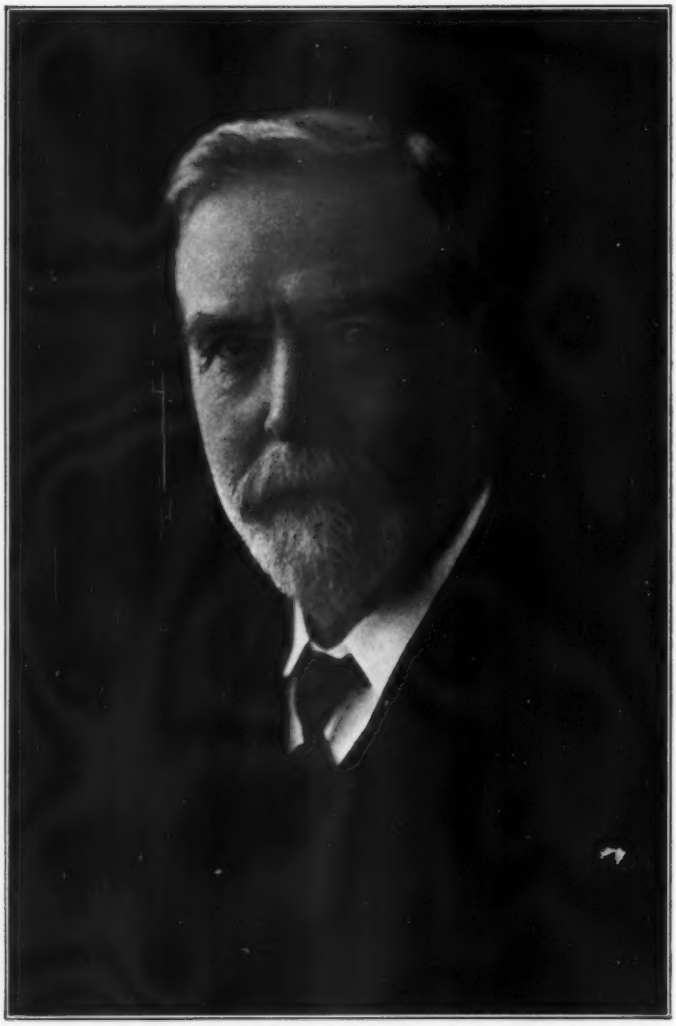

Sir Joseph Cooke Verco

Joseph Cooke Verco, who endowed this Journal, died on July 29th, within a few hours of his eighty-second birthday. Born and brought up in Adelaide, he finished his education as dux of St. Peter's Collegiate School in 1869. He was to have been an engineer, but changed his mind and left, on January 11th, 1870, for London, where he matriculated and became attached to St. Bartholomew's Hospital. His undergraduate career was quite unusually brilliant. He graduated M.B. in 1875, with gold medals in Medicine and Forensic Medicine; M.D. with gold medal in 1876; B.S. with gold medal and a Scholarship in Surgery in 1877. In the same year he became F.R.C.S. After a period as house physician at St. Bartholomew's, he returned to Adelaide in 1878, and quickly developed a lucrative and responsible practice. Each year added more responsibilities, and in quick succession he became Honorary Physician at the Children's Hospital (1879), Honorary Physician at the Adelaide Hospital (1880), joint lecturer in Medicine and Therapeutics in the newly established Medical School in the University (1887), and in the same year, when thirty-six years of age, he was given the greatest honour that the medical profession of Australia could confer, the Presidency of the first Intercolonial Medical Congress of Australia. Two years later he became Dean of the Medical Faculty.

He was an ideal teacher; a good voice, meticulously arranged and selected subject matter, and a happy gift of creating interest, made him outstanding as a lecturer. In the wards his precision and greatness as a clinician had to be seen to be appreciated. There can be no doubt that Verco was responsible for the keen clinical sense that has characterized this Medical School. It seems a contradiction that so methodical and accurate an observer was a believer in "a clinical intuition"; and yet it was so. He felt his responsibility as a teacher keenly. "How responsible is the duty of teachers to communicate the right kind of information, and to beget and stimulate that manner of working which will enable men to utilize their subsequent experience; and not only to hold fast to that which they have, but to grow in wisdom and knowledge, instead of degenerating into the lazy empiricism of a graduated quackery." Speaking in 1891 of the influence of medical students on the efficiency of the Hospital: "My experience is doubtless shared by all my fellow-teachers, that while we teach others a little, we teach ourselves much, and are conscious of a self-improvement. . . . The Medical School has, without the shadow of a doubt, raised the scientific tone of the Hospital, and, unlikely as it may seem, the medical student is an inspiration."

His interest in the medical student went far beyond his routine teaching. He was President of their Society for many years, and, to recall old times, as he used

to say, he kept as complete a record as was possible of every student he had taught. Long after passing from the medical school, his old pupils knew they could put their difficulties to him without fear; and no one that did so ever came away without the best that Joseph Verco could give.

After his retirement from active teaching, he continued as a member of the Faculty of Medicine until 1928, when ill-health forced him to resign all his positions. He had been for forty-one years a member of this Faculty. It was due to his influence that the Darling Laboratories were established. The erection of the Dental Hospital after the War was at his suggestion, and he became Dean of the Dental Faculty from 1921-1928. For sixteen years he was a member of the controlling body of the University.

Outside the University his influence was no less felt. He was President of the Royal Society of South Australia from 1903-1921, and from 1914 until his death he was Honorary Curator of the Conchological Section of the South Australian Museum.

It is a tribute to the orderliness of his mind and the stamina with which he was endowed, that these works were done largely while he was in the active practice of his profession. Yet I doubt if anyone ever saw him in a hurry. He had extreme clarity of thought; everything he did was accomplished step by step, each given due consideration, so that wasted effort took little part of his life. He had, moreover, a well-developed critical faculty and a rare sense of justice. There was no taint or tincture of the mercurial in him, and a straightforward, charming personality was completed by a quiet but keen sense of humour.

Among the numerous benefactions which he made, that to this Journal of £5.000 is one of the few that became largely known. It was his habit to disburse quietly among charities and other objects which had his sympathy what remained from a considerable income after his own simple wants had been supplied.

He had firm spiritual beliefs, without which he felt life was incomplete. Even through "the infinitely sad and transcendently difficult disease of the mind," he believed that we are "led on to a recognition of the spiritual in man and to a wider and deeper conviction that spiritual health is essential to a fullness of the physical."

His writings were mainly conchological, and appeared in the Proceedings of the Royal Society of South Australia. His main contribution to medical literature was in collaboration with the late Sir Edward Stirling in Allbutt's System of Medicine, on Hydatid Disease. The Australasian Medical Gazette, and the published papers of the Australian Medical Congresses, contain many excerpts from his experience.

His health commenced to fail during a trip to England in 1923, when he unfortunately contracted influenza, which affected his heart. Shortly after he was eighty he survived with inimitable equanimity an attack of lobar pneumonia. At a moment when his life was in extreme peril, it was characteristic of him that

he should have said to his friends who were with him: "In case I cannot thank you later, I would like to do so now." It exemplified his fortitude and cheeriness through several years of illness, in which he was tended by Lady Verco with a devotion that was a beautiful counterpart of his own firmness of purpose.

He was created Knight Bachelor in 1919. Verco Ward at the Adelaide Hospital, and the Verco Medal of the Royal Society of South Australia, are two recognitions which his associates in Adelaide were able to give him.

The photograph shows him in later years. It was taken twelve years ago.

W.R.

A NOTE ON THE USE OF FEULGEN'S REACTION AS A CRITICAL STAIN FOR WHEAT CYTOLOGY

by

H. C. GURNEY

(From the Waite Institute for Agricultural Research, University of Adelaide).

(Submitted for publication 6th February, 1933.)

The discovery by Feulgen and Rossenbeck (1924) of a deep purple staining reaction occurring between nucleic acid of the thymo-nucleic type, hydrolyzed with dilute acid and fuchsin-sulphurous acid, has provided a stain which has the advantage over Heidenhain's haematoxylin in that it requires no differentiating.

Ludford (1928) has described the preparation of the stain and the technique of the reaction for animal tissues; and Margolena (1932) has modified it to suit a variety of plant tissues. Her researches, and the chemical investigations of Lison (1932), showed that the reaction was not specific for thymo-nucleic acid.

Although its value as a critical test for chromatin is not as great as was previously thought, the reaction is exceedingly valuable for the cytological staining of chromatin in all stages. Blunt (1932) has used it in a study of the structure of the chromosomes of *Zea mays*, and both Ludford (1928) and Margolena (1932) report excellent staining of chromosomes. Nucleoli never stain, thus proving them essentially non-chromatic.

The details of the preparation of the stain have been fully described by both Ludford (1928) and Margolena (1932). Lison (1932) has shown that the essential staining substance is a combination of SO_2 with parafuchsin leuco-sulphonic acid, which only gives the staining reaction in the presence of excess SO_2. Therefore the final staining solution should smell quite strongly of SO_2.

The nucleic acids in the sectioned material have to be weakly hydrolyzed by means of dilute HCl prior to staining. Substances, presumably of an aldehydic nature, are produced, which then form a deep purple additive compound with the stain. The correct amount of hydrolysis is important. Accordingly sections of wheat anthers and of wheat root-tips, cut at 10 μ and fixed to slides, were subjected to the following hydrolytic treatments, at 60°C.:

1.	3 Normal	HCl	for 5	minutes
2.	2 Normal	HCl	„ 5	„
3.	Normal	HCl	„ 10	„
4.	0·5 Normal	HCl	„ 10	„
5.	0·25 Normal	HCl	„ 10	„
6.	0·25 Normal	HCl	„ 5	„
7.	0·125 Normal	HCl	„ 5	„
8.	0·125 Normal	HCl	„ 3	„

The most brilliant staining resulted from hydrolyzing with normal acid for 10 minutes at 60°C. The influence of temperature was tested out by using normal acid for 10 minutes at 40°, 50°, 60°, 70°, and 80° C. The best results were obtained at 60°C. Hydrolyzing at 60°C. for longer periods, up to 25 minutes, did not improve results; in fact, after 15 minutes the results seemed inferior.

Care should be taken when transferring the slides from hot to cold acid to prevent the sections from floating off the slide. It was found safest to cool the hot acid slowly by immersing the vessel in running water, and then to transfer the slides to cold acid. After rinsing in distilled water the slides were placed in the staining fluid. Two hours proved ample for staining.

Once the technique is standardized for any particular material it requires little supervision, and large numbers of slides may be stained at once. The staining is molecular, not diffuse; consequently it is not possible to overstain. Of the substances present in a dividing plant or animal nucleus, only chromatin will stain. All stages, from the slender spireme to the thick bivalent chromosomes, stain a deep purple or a light purple, according to their chromaticity, and one has a perfectly stained picture of whatever meiotic stages may occur on the slide. The edges of chromosomes are clear-cut and distinct, constrictions and splits are easily seen, and chromosomes which may be bunched together or overlying may be more easily distinguished under high power.

The purple colour of the stain is only seen in daylight. In artificial light the colour appears a brilliant red. With artificial light and a green Wratten filter —B the chromatin appears a velvet black on a light green background.

As far as the actual brilliance of the staining is concerned, no difference was observed after the various fixatives used. These were (1) Carnoy's, (2) Sat. $HgCl_2$ with 2% acetic acid, and (3) chrom-formalin, 50 parts of 1% chromic acid solution to 50 parts of 10% formalin. Better fixation of the wheat pollen mother cells was obtained with (1) and (3) than with (2). The same concentration of acid and time of hydrolysis, as outlined above for the staining of wheat chromosomes, gave equally good preparations when used with the various species of *Phalaris*.

The clear definition of the chromatin persisted under high powers of magnification, the early prophase stages of the meiotic division in particular being much more clear-cut than is usually found with haematoxylin. It is also satisfactory to know that chance artefacts and inclusions will not stain. Cell walls, nuclear membrane, cytoplasm, spindle-fibres, and nucleoli remain unstained.

Delicate strands of chromatin, connections between chromosomes, and even in some cases the chromonema-thread, were well defined. At meiotic metaphase the tetrad nature of the chromosomes is well seen, and clear pictures for counting purposes are obtained in polar view.

I am indebted to Mr. K. C. Richardson, of the University of Sydney, for calling my attention to the reaction and the literature.

REFERENCES.

Blunt, G. M. (1932): Science, 76, p. 372.
Feulgen and Rossenbeck (1924): Z. Phys. Chem., 135, p. 203.
Lison, L. (1932): Bull. D'Histologie Appliquée, 9, p. 177.
Ludford, R. G. (1928): Proc. Roy. Soc., B, 102, p. 397.
Margolena, L. A. (1932): Stain Technol., 7, No. 1.

THE EFFECT OF NITRATE SUPPLY ON TRANSPIRATION RATIO IN PLANTS

by

L. A. T. BALLARD

(Waite Agricultural Research Institute, University of Adelaide).

(Submitted for publication 21st June, 1933.)

INTRODUCTION.

It has been known for many years (Wilfarth, 1905; Crist, 1926; Trumble, 1932) that the ratio of the amount of water transpired during the life period to the amount of dry matter elaborated, or transpiration ratio, of plants abundantly supplied with nitrate is smaller than that of plants with a lower nitrate supply. Although nitrate supply is known to have a pronounced influence on growth and metabolism, the physiological interpretation of its effect on transpiration ratio is still obscure; it is therefore proposed in the present work to analyse this effect and endeavour to elucidate it in terms of the physiology of the plant.

THEORETICAL CONSIDERATION OF TRANSPIRATION RATIO.

In this section a general expression will be derived relating transpiration ratio to the drifting size and rate of water loss of the plant during its life-period. In the light of existing data we may then consider the effect of nitrate supply on the components of the expression, and also seek for possible mechanisms by which these effects may be produced.

If y = total amount of water lost between zero time and any other time t, W = dry weight at time t, and T = value of t in days at harvest,

$$\text{transpiration ratio} = \frac{y_T}{W_T};$$

also

$$\frac{dy}{dt} = rW,$$

where r = rate of transpiration per unit dry weight of plant, and is a function of t.

Therefore

$$y_T = \int_0^T rW\,dt,$$

and

$$\frac{y_T}{W_T} = \int_0^T r\frac{W}{W_T}\,dt \quad .. \quad .. \quad .. \quad .. \quad (1)$$

The quantity $100\ W/W_T$ is the percentage of the maximum dry weight attained at any instant. This percentage and the transpiration rate, r, are thus determinants of transpiration ratio.

Petrie (1932) has considered the case of two plants, having the same rate of transpiration per unit weight but growing at different rates, and has indicated that, if harvesting is deferred till both attain the same dry weight, the slower growing plant will have transpired less water and will hence have a smaller transpiration ratio. Two plants treated with different amounts of nitrogen compounds should behave in an analogous manner; for Rippel and Meyer (1933), using ammonium sulphate, have shown that at any time prior to that of final harvest the percentage of the maximum dry weight attained is decreased as nitrogen supply increases up to a certain point. The consequent reduction in $\int_0^T \frac{W}{W_T} dt$ will contribute to a reduction in transpiration ratio.

Nitrate may influence the other component, transpiration rate, by means of changes in the morphology of the plant or in the physical and chemical systems involved.

Let us consider the morphological changes first. Qualitative observations suggest that plants with a larger nitrate supply are more leafy, and from this relative increase in the transpiring surface an increase in the transpiration rate per unit of plant may be expected.

Gregory and Richards (1929) report a reduction in the ratio of leaf area to dry weight of leaves under conditions of nitrate deficiency, so we may expect an increase in this ratio with increasing nitrate supply, and as before an increase in the transpiration rate per unit of plant.

It has been generally found that the ratio of shoot to root increases with nitrogen supply; this has been shown with nitrate (Turner, 1922), and with ammonium sulphate (Rippel and Meyer, 1933), and it might be thought that the relatively decreased root development would decrease the water supply, and so restrict transpiration. A necessary corollary to this, however, is a reduced water content of the plants, whereas it is known that nitrates cause the water content to increase (Crist, 1926; Pearsall and Ewing, 1929). The altered ratio of shoot to root does not, therefore, directly influence the transpiration rate.

Changes in leaf anatomy might be expected to be most potent in affecting transpiration rate. Pearsall and Ewing quote Miss Ryan as having found no constant differences in frequency of stomatal aperture as a result of nitrate treatment; Mothes (1932), on the other hand, finds that nitrogen-deficient plants differ from normal in having greater cuticular development, more stomata per unit area without any reduction in size, a greater length of vascular tissue, the vessels of which are wider, and a greater internal cell surface. Most of these changes would be expected to lead to an increased transpiration rate; but actually Mothes has observed a decreased rate. This he attributes to the operation of a "transpiration resistance" in the deficient plants as their water content falls.

The first of the physical and chemical changes to be considered is the increase in osmotic pressure of the external solution associated with the addition of nitrate (or any other salt), and Meyer (1931), who noted a decrease in transpiration rate under these conditions, seeks an explanation in these terms. There are two possibilities to be considered when the osmotic pressure of the external solution is increased. The first is that the concentration of solutes in the cell sap remains unchanged; the suction pressure of the cells will then be reduced, and the water content of the plant will fall. It is unnecessary to consider the result of changes in imbibitional capacity of the colloids because of this, since the reduced water content is contrary to observation; hence osmotic changes cannot affect transpiration rate by this means. The second possibility is that the concentration of solutes in the cell sap rises; the suction pressure will then be less reduced than in the previous possibility, and this will tend to prevent a falling water content. If the internal osmotic pressure increases more than the external, the suction pressure will become higher than before, leading to increased water content as observed. Records differ on the effect of nitrogen on the osmotic pressure of the cell sap. Mothes states that the sap of nitrogen-deficient plants has a higher osmotic pressure, due possibly to an accumulation of soluble organic compounds as a result of inefficient synthesis. Eaton (1927) and Petrie (1932) have found that treatment with nitrate increases the osmotic pressure of the expressed sap. However, the latter author has shown that the changes involved are not of sufficient magnitude to affect appreciably the vapor pressure of the sap, so the transpiration rate will not be significantly altered.

Pearsall and Ewing (1929) observed an increase in water content of plants abundantly supplied with nitrate, as well as a reduction in transpiration rate, and attribute these phenomena to an observed decrease in the hydrogen-ion concentration of the cell sap. They conclude that the protoplasmic proteins, normally on the alkaline side of their isoelectric points, will be still further dissociated, and, as an increase in the swelling capacity of the proteins is associated with such a change, they suggest that this underlies the increased water content and decreased transpiration rate.

There is also the possibility that the nitrate ion may influence the permeability of the plasma membranes of the mesophyll cells to water, and since water must pass through these membranes before evaporating from the cell walls into the intercellular spaces, it might be expected that transpiration rate would be affected also.

There is clearly some uncertainty about the effect of nitrate on the transpiration factor, and while the effect of ammonium sulphate on the growth factor has been shown, the effect of nitrate has yet to be investigated. In the following pages an account is given of two experiments in which plants were grown under various conditions of nitrate supply, and by which the parts played by the various factors governing the transpiration ratio have been examined.

MATERIALS AND METHODS.

On 16th January, 1933, selected seeds of Sudan grass (*Andropogon sudanensis* Leppan and Bosman) were set to germinate over distilled water. On the 20th and 23rd uniform seedlings were transplanted into 90 glazed pots which had been filled with 14 kg. of water-washed sand. Nutrient solutions were added on the 26th and 27th, so that each pot received the following amounts:

KH_2PO_4	0·60 grm.
K_2SO_4	2·00
$MgSO_4.7H_2O$	0·40
$CaCl_2$	0·88
$FeCl_3$	0·06
$MnSO_4.4H_2O$	0·0016
$NaNO_3$	0·75

On the 31st, the seedlings were thinned to three uniform plants per pot, and the following day one-third of the pots received 1·5 grm. of $NaNO_3$ in solution, and another third 3·75 grms. of $NaNO_3$, thus subjecting the plants to three treatments, to be referred to as:

Treatment	I	receiving	0·75	grm.	of $NaNO_3$
Treatment	II	„	2·25	„	„ „
Treatment	III	„	4·50	„	„ „

Finally water was added in such amount as to raise the moisture content of the sand to 57% of its maximum water-holding capacity, and weekly transpiration losses were recorded by weighing the pots and bringing back to their previous weights by further addition of water. One and a half kilogrammes of fine gravel were spread on the surface of each pot to restrict evaporation, and losses due to the latter process were allowed for by leaving six similarly treated pots to fallow. Transpiration losses became appreciable on 8th February.

On the 13th and 23rd February, 13th March, 4th April, and 2nd May, 6 replicates of each treatment were harvested. Exsertion of panicles first occurred on the 17th March, and flowering commenced on the 23rd, so that at the time of harvest 4 the grain was in the pre-resting stage, and in the resting stage at the final harvest.

At each harvest the leaves were separated from the stems to obtain an approximate measure of the transpiring portion of the plant. Only living laminar tissue was included in the leaf portions, the leaf sheath, and in the later harvests, the dead leaves and withered tips as well as the inflorescences, being included with the stem. The material was dried to constant weight at 100°C.

The second experiment was carried out on barley plants, using the same general technique as before, except that the nutrients other than $FeCl_3.6H_2O$ and $MnSO_4.4H_2O$ were applied before planting, and the moisture content of the sand was 50% of its maximum water-holding capacity. Each pot received the following amounts of nutrient salts in solution:

KH_2PO_4	0·60 grm.
K_2SO_4	1·30
$MgSO_4.7H_2O$	0·40
$CaCl_2$	1·05
$FeCl_3.6H_2O$	0·10
$MnSO_4.4H_2O$	0·0016

Solutions of $NaNO_3$ were added so that pots under different treatment received 0·33, 0·66, 1·32, and 2·64 grms. of this salt respectively, and the same quantities were given to the remaining pots of each treatment after the first harvest.

On the 30th May, 1932, 3 groups of 3 seeds of a pure line of Cape barley were planted in each pot, and these were later thinned to 3 uniform plants per pot. A severe infection with mildew occurred, and in some cases one plant in a pot died, or its growth was markedly affected, and in such cases the results of the pot were rejected.

On the 1st August, 5th September, 3rd October, and 24th October, 6 replicates of each treatment were harvested. Exsertion of anthers had occurred about a week prior to harvest 3, and the grain was still in the pre-resting stage at the last harvest, which was thus more comparable to harvest 4 of the previous experiment.

At each harvest the material was cut finely and the sap expressed from it under a pressure of ½ ton per square inch. The dry weight of the plants from each pot was determined by measuring the volume of sap expressed, and drying a portion of it at 100°C., as well as the material remaining in the press after each extraction.

Hydrogen-ion concentration of the expressed sap was determined, combining the sap from 3 pots of each treatment in harvests 1 and 4, and from 2 pots in harvests 2 and 3. The *p*H determinations were made by means of a shaking hydrogen electrode using a Clarke electrode vessel. Readings were taken till constant potential was obtained (usually 10–15 minutes were necessary for this), and the apparatus was frequently checked against a phthalate buffer solution.[1]

RESULTS.

The results of the Sudan grass experiment are summarized in Table 1, which presents the mean dry weights, the water contents expressed on a dry weight basis, the amounts of water transpired, and transpiration ratios in c.c. of water transpired per grm. of dry matter produced. The table also gives for each treatment at the several harvests the ratio of leaf weight to the weight of the whole plant, which has been called the leaf weight ratio by Inamdar, Singh, and Pande (1924). The leaf weight ratios of this study, however, differ from those of Inamdar, Singh, and Pande in that only living leaf material is included in the numerator, and root material is excluded from the denominator.

The most pronounced effect of treatment has been to increase the final dry weight markedly. Reference to fig. 1, however, shows that the plants passed through an initial phase, during which their size was a decreasing function of their nitrate supply; subsequently plants of Treatment II became larger than those of Treatment I; and later still plants of Treatment III grew larger than those of Treatment I, and finally, at a comparatively late stage in the life-period, larger than those of Treatment II. Until flowering, the water content of the plants followed a somewhat similar course.

Transpiration ratio, up to the time of harvest 4, has been reduced 7–11% by treatment, and has been increased at the final harvest.

Analyses of variance have been performed on the transpiration ratios to test the significance of the reductions at harvests 2, 3, and 4, and the increase at harvest 5. In these analyses the variances due to position in the glass-house[2]

[1] The thanks of the author are due to Mr. H. C. Trumble for advice on matters concerned with the growing of the plants, and to Mr. R. J. Best for advice on the *p*H determinations.

[2] The pots were arranged in rows of 6 replicates of each treatment transversely across the glass-house, the treatments being repeated recurrently at right-angles to this. At harvest 3 adjacent rows of 6 pots were serially removed. Thus any differential effects due to longitudinal position were grouped with the environmental changes between harvests, while the variations within a set of pots reserved for one harvest were allowed for in the manner indicated in the text.

have been subtracted from the total variances, and the z values for treatment : remainder, as well as standard deviations, are given in Table 2. The high standard deviation at harvest 2 may possibly be due to the fact that the total water losses were extremely small and their values derived from only 2 weekly weighings. Although the increase in transpiration ratio values at harvest 5 is not significant, it is clear that there must have been an increase during the interval between harvests 4 and 5, since there had been a very significant reduction at harvest 4.

TABLE 1.

Mean Values of Dry Weight, Percentage Water, Water Loss, Transpiration Ratio, and Leaf Weight Ratio in Sudan Grass.

	Treatment.		
	I	II	III
Harvest 1.			
Dry weight (grms.)	0·584	0·544	0·416
Percentage water	764·9	745·3	706·4
Leaf weight ratio	0·641	0·640	0·626
Harvest 2.			
Dry weight (grms.)	3·23	3·09	2·36
Percentage water	628·1	751·5	702·5
Water loss (litres)	1·02	0·92	0·69
Transpiration ratio	**315**	**297**	**293**
Leaf weight ratio	0·583	0·604	0·625
Harvest 3.			
Dry weight (grms.)	13·55	22·84	20·28
Percentage water	438·5	457·8	550·1
Water loss (litres)	3·76	5·59	5·12
Transpiration ratio	**277**	**245**	**253**
Leaf weight ratio	0·325	0·336	0·424
Harvest 4.			
Dry weight (grms.)	20·31	44·01	47·81
Percentage water	304·1	278·5	319·7
Water loss (litres)	4·82	9·75	10·39
Transpiration ratio	**238**	**222**	**217**
Leaf weight ratio	0·125	0·120	0·191
Harvest 5.			
Dry weight (grms.)	24·92	47·09	59·29
Percentage water	209·8	196·0	232·3
Water loss (litres)	5·96	11·50	14·82
Transpiration ratio	**240**	**244**	**251**
Leaf weight ratio	0·0347	0·0377	0·0829

The results of the second experiment are given in Table 3. Here also, increasing nitrate supply has increased the final dry weight and the water content. In this case a reversal of order of dry weights similar to that observed on the Sudan grass is not revealed by the data presented; but such a reversal was de-

tected visually and by the weekly transpiration data, although it was completed by the first harvest. The data are not subjected to the same analysis as in the previous experiment; but they confirm the fact that the transpiration ratio has fallen with increasing nitrate supply.

TABLE 2.

Analyses of Variance for Transpiration Ratios in Sudan Grass.

Harvest	z value Treatment : Remainder	Standard Deviation	Standard Deviation %
2.	0·6348	14·8	4·9
3.	1·4912	9·3	3·6
4.	1·4565	6·1	2·7
5.	Treatment variance < remainder variance	15·3	6·1

In all cases the z value at the 5% point = 0·7058, and at the 1% point = 1·0114.

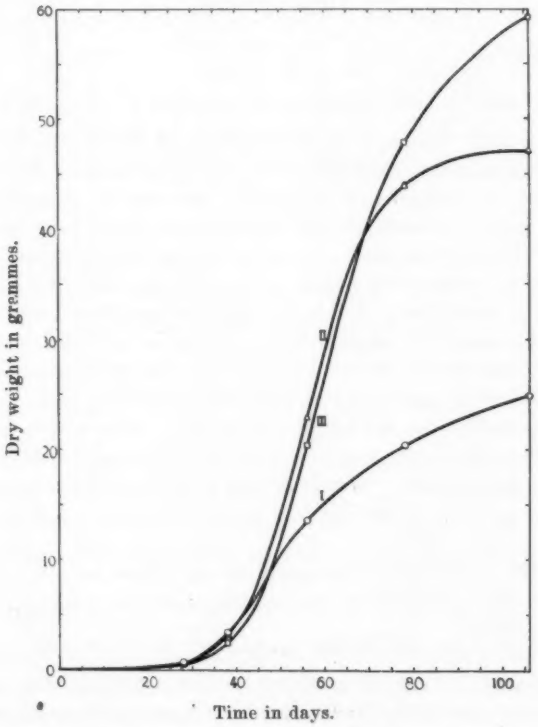

Fig. 1. Growth curves of Sudan grass.

It is interesting to note that transpiration ratio drifts in a similar manner in the two experiments. In both cases the value for the transpiration of the highest treatment rises above the values of the intermediate treatments at the third harvest, which took place about a week before flowering in the Sudan grass experiment and about a week after in the barley experiment, and falls at the fourth harvest. Although the probability that such a rise at harvest 3 could be obtained by chance is 30% in the Sudan grass experiment, as adjudged by Fisher's t test, it is $<1\%$ in the barley experiment.

The hydrogen-ion concentration of the expressed sap of the barley decreases with increasing nitrate supply, as was found by Pearsall and Ewing (1929). It may also be seen that the hydrogen-ion concentration rises steadily till the time of flowering, when it falls again. It is interesting to note in this connection that Hurd (1924) found a progressive increase in hydrogen-ion concentration in the expressed sap of wheat through the whole life-period.

DISCUSSION.

The Growth Effect.

Previously it had been indicated that the quantity $\int_0^T 100 \frac{W}{W_T} dt$ is a determinant of transpiration ratio. In figs. 2, 3, and 4 curves of $100\ W/W_T$ for harvests 3, 4, and 5 of the Sudan grass experiment are plotted against t. Extra points have been obtained by interpolation on the growth curves of fig. 1, so that the assumptions made in drawing smooth curves through the points have been maintained consistently. Values of the same expression for the barley experiment are given in Table 4. At any point prior to that of harvest, the ratio of the amount of dry matter produced after that point, to the amount produced before it, is in most cases greater the larger the nitrate supply. This is in accord with the observations of Rippel and Meyer (1933), and the fact that these workers supplied the nitrogen as ammonium sulphate precludes the possibility that the effect we are investigating is due to the Na-ion of the $NaNO_3$. One factor contributing to this state of affairs is the prolonged growth of the younger tillers of the plants with a high nitrogen supply. However, this is not the only factor possible, and a more detailed analysis of the effect of nitrate on growth will be attempted on another occasion.

To assess the reduction in transpiration ratio this growth effect could produce, assuming that transpiration rate is independent of time and treatment, the ratios of $\int_{23}^T 100 \frac{W}{W_T} dt$ for the various treatments have been obtained by measuring the areas under the curves in figs. 2, 3, and 4 between the required dates. Since we are to compare these values with those actually observed, the lower limit is day 23 in all cases, as this was the day on which transpiration became appre-

ciable and was first measured. In Table 5 the observed values are compared with these theoretical values by adopting Treatment I as a standard. It is clear that with one exception the growth of the plants is such as to cause a decrease in transpiration ratio throughout, and this decrease is greater than that actually observed.

TABLE 3.

Mean Values of Dry Weight, Percentage Water, Water Loss, Transpiration Ratio, and pH of Expressed Sap in Barley.*

	Treatment.			
	I	II	III	IV
Harvest 1.				
No. of replicates	6	6	6	6
Dry weight (grms.)	1·60	2·21	3·07	3·65
Percentage water	680·0	793·3	920·1	1020·8
Water loss (litres)	0·62	0·79	1·01	0·98
Transpiration ratio	**387**±37·6	**360**±14·8	**329**±20·1	**269**±7·8
*p*H of sap	6·02	6·01	6·22	6·27
Harvest 2.				
No. of replicates	6	6	4	6
Dry weight (grms.)	4·76	7·41	11·76	12·88
Percentage water	449·5	489·7	550·7	661·1
Water loss (litres)	1·55	2·32	3·61	4·14
Transpiration ratio	**327**±25·9	**311**±2·7	**308**±10·8	**322**±17·4
*p*H of sap	5·84	5·92	5·96	5·98
Harvest 3.				
No. of replicates	5	5	4	5
Dry weight (grms.)	10·26	14·76	27·87	30·01
Percentage water	248·4	265·8	286·8	365·2
Water loss (litres)	3·10	4·23	7·88	8·66
Transpiration ratio	**302**±17·1	**288**±15·5	**274**±10·0	**288**±5·2
*p*H of sap	5·84	5·84	5·90	5·94
Harvest 4.				
No. of replicates	6	6	6	3
Dry weight (grms.)	13·92	22·21	37·96	52·77
Percentage water	173·0	180·5	195·0	196·3
Water loss (litres)	4·27	6·25	10·54	13·22
Transpiration ratio	**307**±14·1	**282**±9·9	**278**±3·8	**251**±11·5
*p*H of sap	6·00	5·99	6·06	6·05

* Transpiration ratios given with standard deviations, $\sigma = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$

Transpiration Rate.

The values obtained for the reduction in transpiration ratio that would be obtained by the growth effect alone are liable to error, due to inaccuracy of figs. 2, 3, and 4, resulting from the infrequency of points. It is unlikely, however, that the error is of such magnitude as to invalidate the conclusion that the possible

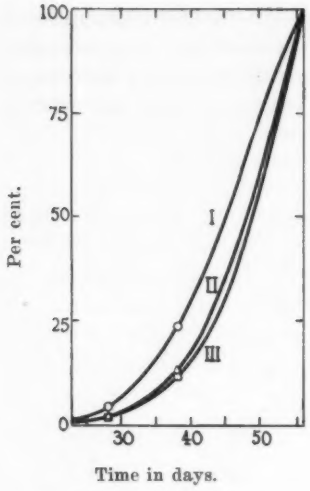

Fig. 2. Curves showing the drift of percentage dry weight. For each treatment the dry weight is expressed as percentage of that attained at harvest 3 (Sudan grass experiment).

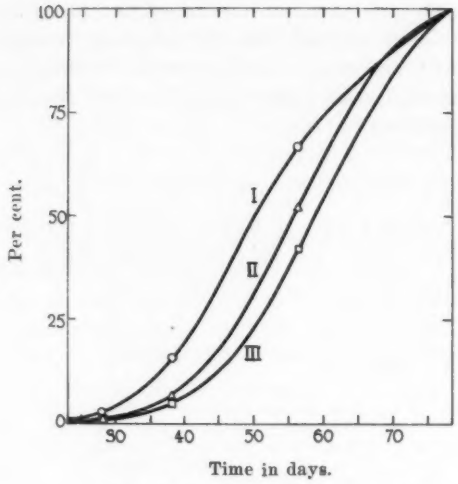

Fig. 3. Curves showing the drift of percentage dry weight. For each treatment the dry weight is expressed as percentage of that attained at harvest 4.

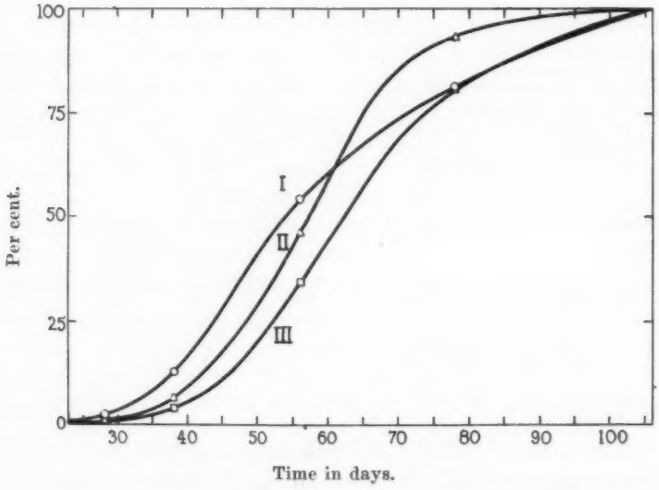

Fig. 4. Curves showing the drift of percentage dry weight. For each treatment the dry weight is expressed as percentage of that attained at harvest 5.

effect is too large. The other determinant of transpiration ratio, transpiration rate, must therefore operate in the reverse direction. This could be achieved either by a rise in transpiration rate with age, which would have the effect of weighting the transpiration losses when the differences in percentage size were least marked, or by an increase of the transpiration rate with treatment. This effect of treatment might be independent of time, or it might be of such a nature as to cause a differential time drift of transpiration rate; if the effect increased with time it would contribute still further to an increase in transpiration ratio.

TABLE 4.

Dry Weights of Barley attained at Harvest 1 expressed as Percentages of those at Harvest 2.

Harvest.	Treatment I.	Treatment II.	Treatment III.	Treatment IV.
1.	33·61	29·83	26·11	28·34
2.	100·00	100·00	100·00	100·00

Dry Weights attained at Harvests 1 and 2 expressed as Percentages of those at Harvest 3.

Harvest.	Treatment I.	Treatment II.	Treatment III.	Treatment IV.
1.	15·60	14·97	11·01	12·16
2.	46·40	50·20	42·19	42·92
3.	100·00	100·00	100·00	100·00

Dry Weights attained at Harvests 1, 2, and 3 expressed as Percentages of those at Harvest 4.

Harvest.	Treatment I.	Treatment II.	Treatment III.	Treatment IV.
1.	11·49	9·95	8·09	6·92
2.	34·20	33·37	30·98	24·41
3.	73·69	66·45	75·41	56·86
4.	100·00	100·00	100·00	100·00

The transpiration rates of the Sudan grass have therefore been obtained in the following manner. From the weekly values of transpiration per pot have been calculated the average weekly transpirations of the six pots of each treatment for the harvests under consideration, and from these the cumulative water losses. These have been plotted to a large scale and the points joined by smooth curves, presented much reduced in figs. 5, 6, and 7. From these curves water losses for 2-day periods have been obtained by interpolation. By means of these values smoothed curves of the rate of water loss per 2 days per pot have been drawn (figs. 8, 9, and 10). The general shape of the curves of cumulative water loss and rate of water loss per 2 days is similar for the six pots of any one treatment harvested at different times, and the actual differences may be attributed to position in the glass-house. The transpiration rates at harvests 3, 4, and 5 have then been derived from the water losses for 2-day periods and dry weights at the times of these harvests. Transpiration rates at harvest 2 have been derived from the

means of 3 values each obtained from the dry weights at harvest 2, and the water losses for 2-day periods at that time, of the six pots taken at harvests 3, 4, and 5 respectively.

TABLE 5.

Observed and Derived Transpiration Ratios.

	Treatment. I.	Treatment II.			Treatment III.		
Harvest.	Observed.	Observed.	Derived.	*Rp**	Observed.	Derived.	*Rp*
3.	277	245	247	215	253	257	201
4.	238	222	225	203	217	223	178
5.	240	244	248	245	251	256	212

* *Rp* = Transpiration ratios derived from $\int_{23}^{T} \frac{W}{W_T} dt$ assuming transpiration rate independent of time and treatment.

These values are given in Table 6, which shows that the transpiration rate per unit dry weight of plant falls with time throughout the greater part of the experiment, tending to follow the drift in leaf weight ratio. This will cause even further reductions in transpiration ratio. The magnitude of the reduction in transpiration ratio that the drift in transpiration rate would cause has not been determined quantitatively, since the transpiration rates early in the life-period cannot be obtained with sufficient accuracy.

The reductions caused by the growth of the plants and the drift in the transpiration rate are partially compensated for by an increase in transpiration rate per unit of plant with treatment. This may be due to an increase in the real rate of transpiration per unit of leaf, or merely to a relative increase in the transpiring surface. As Table 1 shows the leaf weight ratio to be increased with treatment, part at least of the increase is due to the latter cause. Transpiration rates on a leaf weight basis are also presented. An analysis of variance performed on the 12 observations of Table 6 shows treatment to have had no significant effect in increasing transpiration rate per unit of leaf, the z value for treatment : error after subtracting the variance due to time of observation being 0·4777, while it is 0·8188 at the 5% point.

Although the fact that nitrate alters the pH in a manner similar to that observed by Pearsall and Ewing has been verified, no such decrease in transpiration rate as reported by them has been observed. It should be realized, however, that the transpiration rates given in this paper are means of those over periods of 2 days, and furthermore have been obtained from interpolation in the weekly transpiration records, so that we have no knowledge of the minor fluctuations possible.

Of the two factors which have been seen to determine transpiration ratio, the growth effect causes a decrease, with the one exception of Treatment II at

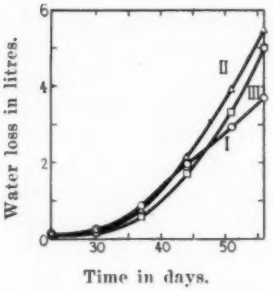

Fig. 5. Curves showing mean cumulative water loss of pots harvested at harvest 3 (Sudan grass experiment).

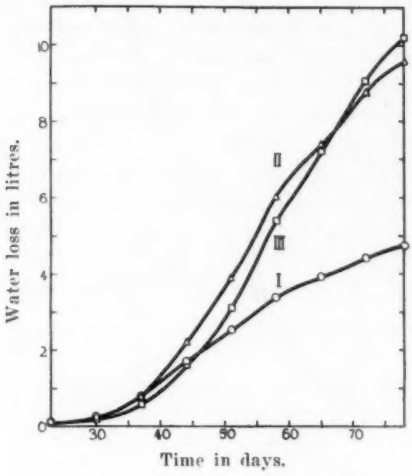

Fig. 6. Curves showing mean cumulative water loss of pots harvested at harvest 4.

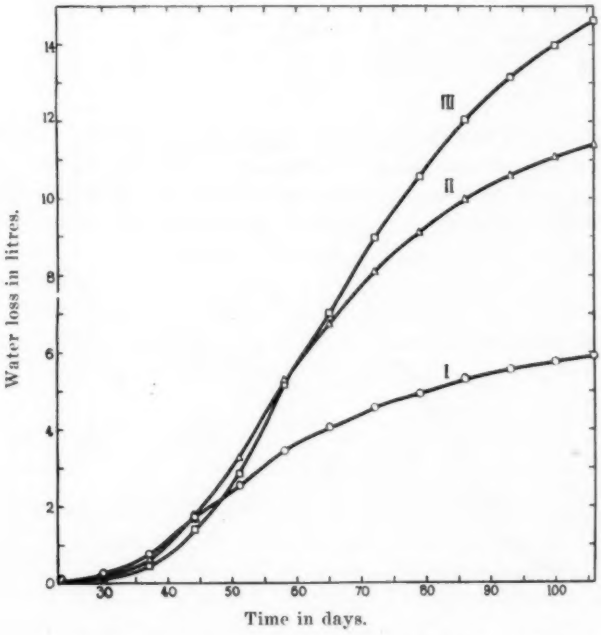

Fig. 7. Curves showing mean cumulative water loss of pots harvested at harvest 5.

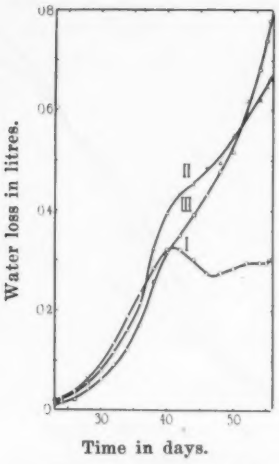

Fig. 8. Curves showing the drift of mean 2-daily transpiration per pot for pots harvested at harvest 3 (Sudan grass experiment).

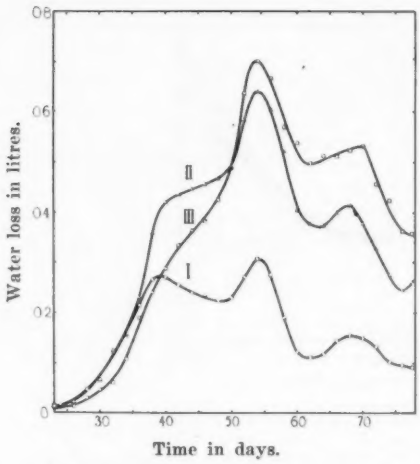

Fig. 9. Curves showing the drift of mean 2-daily transpiration per pot for pots harvested at harvest 4.

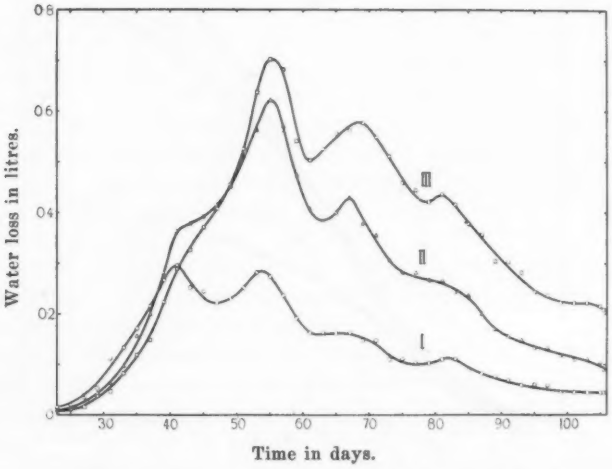

Fig. 10. Curves showing the drift of mean 2-daily transpiration per pot for pots harvested at harvest 5.

harvest 5. This growth mechanism we shall designate A. The transpiration rate factor operates in two ways; the falling with age which contributes to a reduction in transpiration ratio we shall call B, and the increase with treatment which contributes to an increase in transpiration ratio we shall call C. Regarding these mechanisms as depressants of the transpiration ratio, A and B may be designated as generally positive and C as negative. Usually $A + B - C$ is positive. It may, however, be negative, as in the case mentioned above where A is negative, and at harvests 3 and 5 for Treatment III, where C is large. The increase with treatment, C, may have a different magnitude at different times, i.e., B may be affected differentially by treatment, and this would contribute further to the fluctuations observed in the differences in transpiration ratio induced by treatment.

TABLE 6.

Transpiration Rates in c.c. per day per grm. of total Plant, and per grm. of Leaf, at the various Harvests.

	Treatment.					
	I.		II.		III.	
Harvest.	Plant	Leaf.	Plant.	Leaf.	Plant.	Leaf.
2.	40·70	69·88	49·58	82·11	48·21	77·19
3.	11·09	34·11	14·74	43·89	19·72	46·67
4.	2·22	17·67	2·67	22·23	3·70	19·24
5.	0·88	25·91	0·96	25·44	1·74	21·02

Thus all of the changes in transpiration rate per unit of plant, and the effect of treatment on it, are entirely due to the drift of, and the effect of treatment on, the leaf weight ratio. The whole of the effect of treatment on transpiration ratio, whether produced by the mechanisms A, B, or C, is therefore purely a growth phenomenon, and is not concerned with any continued effect on the process of transpiration itself.

An interesting check on the graphical analysis is provided by comparing the observed transpiration ratios with those derived graphically from equation (1). If Q = rate of water loss per pot, at the time t,

$$r = \frac{Q}{W}$$

and from (1)

$$\frac{y_T}{W_T} = \int_0^T \frac{Q}{W_T}\,dt \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Values for the second member of equation (2) between the limits 23 and T have been obtained by dividing the areas under the curves of figs. 8, 9, and 10 by the value of W_T proper to harvest and treatment. The ratios of these values have been compared as before by adopting Treatment I as standard, and are given in Table 5. The values agree to within 3%.

ACKNOWLEDGMENTS.

The author wishes to express his indebtedness to Dr. A. E. V. Richardson for providing facilities at the Waite Institute for this research, part of which was carried out during the tenure of a Henry Gyles Turner Scholarship of the University of Melbourne.

The author is also deeply sensible of his indebtedness to Dr. A. H. K. Petrie for his inspiring advice and generous assistance at all stages of the work.

SUMMARY.

The effect of nitrate in reducing transpiration ratio, which is the ratio of water transpired to dry matter produced up to any point, is due not to an effect on the transpiration rate per unit weight of leaf, but to an effect on growth. This effect is such that, at any point prior to that of harvest, the ratio of the amount of dry matter produced after that point to the amount produced before it is, in most cases, greater the larger the nitrate supply: this causes a reduction in transpiration ratio. The reduction is intensified by the fall with age in transpiration rate per unit of plant, as a result of the fall in leaf weight ratio, for this has the effect of weighting the transpiration losses when the differences in percentage size due to the growth phenomenon are most marked. However, these reductions are partially, and in certain cases more than compensated for by an increase in the transpiration rate per unit of plant, as a result of an increase in the leaf weight ratio.

REFERENCES.

Crist, J. W. (1926): Mich. State College Agr. Exper. Sta. Tech. Bull. 74.
Eaton, F. M. (1927): Amer. J. Bot., 14, p. 212.
Gregory, F. G. and Richards, F. J. (1929): Ann. Bot., 43, p. 119.
Inamdar, R. S., Singh, S. B. and Pande, T. D. (1925): Ann. Bot., 39, p. 281.
Hurd, A. M. (1924): J. Agr. Res., 27, p. 725.
Meyer, B. S. (1931): Amer. J. Bot., 18, p. 79.
Mothes, K. (1932): Biol. Zbl., 52, p. 193.
Pearsall, W. H. and Ewing, J. (1929): Ann. Bot., 43, p. 27.
Petrie, A. H. K. (1932): J. Coun. Sci. and Indust. Res., 5, p. 177.
Rippel, A. and Meyer, R. (1933): Ztschr. Pflanzenernähr, Düngung, A, 27, p. 257.
Trumble, H. C. (1932): J. Coun. Sci. and Indust. Res., 5, p. 152.
Turner, T. W. (1922): Amer. J. Bot., 9, p. 415.
Wilfarth, H. (1905): Bied. Zent. Agric. Chem., 34, p. 167.

THE STANDARD METABOLISM OF THE AUSTRALIAN ABORIGINES

by

C. STANTON HICKS AND R. FRANCIS MATTERS

(From the Department of Human Physiology and Pharmacology, University of Adelaide).

(Submitted for publication 28th May, 1933.)

In a previous paper (1931) we reported the results of gaseous metabolism experiments carried out on aborigines near the head of the Australian Bight. We concluded from our results that climatic factors played a considerable rôle in determining the low values then obtained, and that under-nutrition, and possibly bodily shape, might also be causal factors in the deviations from European standards we obtained.

It was felt that, should opportunity offer, the work should be repeated under true field conditions on the native in a state of nature. At Koonibba the aborigines wore rags of European clothing and were detribalized. The present communication reports the results of measurements of oxygen uptake on individuals belonging to tribes concentrated, as the result of two months' reconnaissance, from the region of Central Australia, west, north, and south of Cockatoo Creek; Cockatoo Creek is situated approximately at Lat. 22° S. and Long. 132° E. The country was drought-stricken, and the natives were not in good condition. They had been living in their natural state, by hunting and by grubbing for roots, and wore no clothes prior to concentration. The Expedition supplemented their diet by issuing rations of boiled wheat and raisins, with an occasional uru (species of kangaroo) or bustard; this was eaten half-roasted. They slept on the ground near their fires, with a few branches of brushwood at their heads to break the force of the prevailing wind. Despite the presence of heaps of smouldering embers of fires interspersed among the sleeping natives, we found the cold of the early morning most unpleasant, even with our many layers of clothing. During the early morning, when the observations on metabolism were made, the temperature ranged from —2°C. to 10°C. The Benedict portable apparatus was again used, and owing to the personal influence of Mr. Kramer, who as an itinerant missionary in these regions was responsible for the concentration of so many natives, we were able to carry out metabolism measurements without a hitch. No hospital patient ever submitted to this type of investigation with such supreme indifference, and our main concern was to see that the native did not fall asleep during

tests. Respiratory and cardiac rates remained unaltered throughout individual observations, which on each native occupied at least three-quarters of an hour, and often exceeded the hour. Three separate periods, during which six measured amounts of oxygen were consumed, form the basis of the results tabulated. Averages of results from the three separate periods were not used, it being assumed that the lowest consistent set of measurements of six volumes of oxygen represents the "standard" value of oxygen consumption. Due care was taken to avoid the error due to over-breathing. The difficulties encountered were mainly due to the size of the mouth and the shape and size of the nostrils of the natives. All results which were not consistent, as determined by Benedict's method (1923) of plotting oxygen consumption against time, have been rejected.

There was a uniform elevation of the metabolism when the results were compared with the calculated values. The obvious explanation is that it was the result of increased heat production under the atmospheric conditions prevailing. The natives did not shiver or show signs of discomfort. They were, in fact, torpid. As the day began to dawn, and our presence led to wakefulness on the part of the other natives, they began to show evidence of being cold, and either huddled together or around a fire, which they replenished with fuel. Whilst asleep, however, or when just awakened, they did not show any signs of being cold, although their body temperatures (mouth and skin) were low (*vide* Table 5). The increased metabolism found at this early and cold period of the morning (5.30 a.m. to 8.30 a.m.), when the camp fires were at their lowest, indicates that energy was being dissipated as heat in order to maintain normal body temperature.

Nos. 64 and 65 were an Arunta police interpreter and his wife. They were thoroughly detribalized, wore clothes, and were covered with blankets. No. 64A was an old police boy, with strong leanings towards the savage state. He wore sparse clothing, and preferred to have no blanket.

That a race of people should so waste their body energy in a country where food is acquired with difficulty is interesting. The low values obtained for oxygen consumption during the hot summer months at Koonibba, and the high values obtained at Cockatoo Creek in the winter, require explanation. Some light has been thrown on the discrepancy by continuous gaseous metabolism measurements, lasting from 6 a.m. to 1 p.m., which have been made at Mount Liebig in August, 1932. The results, details of which will appear in the next communication, show that the standard metabolism falls as the temperature rises.

In order to test the applicability of the surface area formula of Du Bois to these natives, we measured the surface area directly by the method of Takeya (1929), which consists of applying gummed paper strips of known width to the surface of the body; measurement of the length of strip used enabled the area to be calculated. The days were hot, and a strong wind was blowing dust into the tent, and as fast as the gum was moistened it dried in the parching air. The results obtained were under the circumstances gratifying, and they indicate within the limits of 5% a sufficiently close approximation to the calculated values.

It is clear that the shape of these natives is different from those of the region bordering the Australian Bight between Eucla and Fowler's Bay. The tabulated "pelidisi" values show that only 20% exceed 100, as compared with 55% of the Kokotu tribe of the Bight region. The pelidisi value, or relation between the weight and sitting height, was introduced by von Pirquet (1916) as an index of nutrition. Benedict's normal values for men and women are 94 and 96 respectively. Von Pirquet's standard was 98, so that, knowing these natives are not even well fed, it is clear that we are dealing with a bodily conformation which in a variable percentage of cases departs from that of the Western European. Unfortunately, with one exception (No. 64) the direct surface area measurements were made on individuals whose pelidisi approximated closely to the European standard.

Attempts were made to use the flarimeter of Wells and others (1930) to determine vital capacity and to test cardiac response to effort. Our Arunta police-boy interpreters were, however, unable to communicate instructions satisfactorily to these Anmatjera and Ilpirra natives, and after much tedious effort we had to abandon the attempt.

The avidity of the tissues for water was estimated from the rate of absorption of normal saline from the skin, using the method of McClure and Aldrich (1924), one of us, C.S.H., acting as a European control. The tests were carried out at midday, under a hot sun. The three tests on C.S.H. agreed closely, although done at different times, 34 minutes being the average time of absorption. Two intradermal injections of 0·2 c.c. normal saline, 1 cm. apart, were made on the inside of the upper arm midway between the olecranon and axilla, and the duration of the tangible swelling was measured. The valley between the two wheals makes the palpation method more sensitive than when only one wheal is produced. Almost all the values obtained with the natives are smaller than those obtained in hospital practice, the average being $38{\cdot}4 \pm 1{\cdot}2$ minutes. Standard deviation = 6. The control, feeling the heat and being extremely thirsty, owing to evaporation from the skin, gave lower values than the average for the black-skinned aborigine. No striking departure was evident, as might have been anticipated, were the natives' tissues better adapted to retaining water in this environment. Measurements of blood pressure were unfortunately impossible at the time when metabolic determinations were being made. There were only two of us, and the delicate nature of our position in their encampment made it necessary to avoid all complications, such as might have been introduced by requiring subjects to remain longer than the time required for the more important investigation. The measurements of pressure were therefore made during the day, as opportunity offered. The results recorded represent averages for the supine position, since natives had walked in some instances as much as 200 yards to the place where the measurements were made. The uniformly low values obtained indicate that blood pressure of the aborigine would be worth further study. At the moment we can do no more than record, without comment, both these and our

observations on the pulse and respiration. Reference to Table 5 shows that both pulse rates were, on the average, below the normal at the time of the experiment (Table 5). These low rates are associated with a lowered body temperature and a greatly increased gaseous metabolism. Douglas, Haldane, Henderson and Schneider (1913) have show that metabolism increases on standing to approximately 150% of the resting value; while the number of breaths per minute increase from 16·8 to 17·1, and the volume of each expiration from 457 c.c. to 612 c.c. The cardiac rate is slightly increased in the majority of subjects, as a result of the same change of posture, although in many cases there is no rise. The low pulse and respiration rates found in the present investigations indicate that increased tissue oxidation is met by increased cardiac output per beat and increased expiratory volume respectively.

SUMMARY.

The gaseous metabolism of some 40 Australian aborigines of the Anmatjera and Ilpirra tribes of western Central Australia have been measured by the Benedict portable metabolimeter. The natives were unclothed and living in the savage state. They were subject to a variation in the diurnal shade temperature of from —2°C. to +33°C. The oxygen consumption was estimated in the cold of the early morning in the native encampment, 12 hours after the last meal. Without exception the gaseous metabolism was increased in amount varying from 23% to 90%, although there was no indication of shivering and the natives lay as if torpid.

Some remarkably low body temperatures and pulse and respiration rates were encountered. Systolic blood pressure did not exceed 128 mm. Hg even in advanced age.

The direct measurement of the surface area of six natives by the method of Takeya gave results agreeing to within 5% of the values calculated by the Dubois formula.

The rate of absorption of intradermal saline injections did not vary sufficiently from that of the white control to indicate a departure from normal water metabolism.

We have to record our thanks to Dr. T. D. Campbell (leader of the expedition) and Dr. H. Gray for their assistance in making surface area measurements. One of the metabolimeters was lent by the Carnegie Institute of Nutrition, Boston.

REFERENCES.

Benedict and Benedict, F. G. (1923): Boston Med. and Surg. J., 188, pp. 567-577.

McClure, W. B. and Aldrich, C. A. (1924): J. Amer. Med. Ass., 82, pp. 1425-1428.

Hicks, C. Stanton, Matters, R. F. and Mitchell, M. L. (1931): Austral. J. exp. Biol., 8, pp. 69-82.

Mackenzie, L. F., Wells, P. V., Dewis, E. G. and Ylvisaker, L. S. (1930): Amer. J. Med. Sci., 180, pp. 372-385.

Pirquet, C. Freiherr von (1915): Z. Kinderheilk, 14, p. 197.

Cockatoo Creek, August, 1931.

TABLE 1.

Oxygen Consumption and Derived Energy Production at an Oxygen Calorific Equivalent of 4·825 Calories per litre.

No. of Expt.	Minimum O_2 per min.	Calories per hour.	Height.	Weight.	Surface Area Sq. metres.	B.M.R. Cal. sq. m. hr.	Calories per hr. predicted.	Deviation.
64	215	62	166·2	56·75	1·85	33·5	60	+3%
64A	411	118·1	168·6	64	1·75	67·0	65·4	+65%
65	203	58·8	155	48·25	1·45	40·5	54·3	+8%
65A	350	101·4	161·8	63	1·66	61·0	63·4	+59·3%
66	275	79·6	172·0	57·5	1·86	30·9	64·5	+23%
66A	351	101·6	173·0	63	1·74	68·4	63·4	+60·2%
67	342	99·0	172·6	58·6	1·88	52·6	59·5	+66%
67A	352	102·0	163·1	43·5	1·43	71·3	53·3	+91·3%
68	364	105·4	159·1	50·5	1·55	68·0	55·0	+90·9%
68A	371	107·4	171·0	52·0	1·6	67·1	62·3	+72·3%
69A	378	109·4	171·0	54·5	1·63	67·1	59·8	+82·9%
70A	356	90·6	172·3	54·0	1·64	55·2	52·6	+72%
71	320	96·6	169·5	57·7	1·67	57·8	62·5	+54·5%
71A	353	102·2	171·5	56·0	1·65	61·9	53·6	+70·5%
72	280	81·1	162·6	43·2	1·43	56·7	51·4	+57·7%
72A	387	112·0	168·1	56·75	1·63	68·7	59·0	+90·0%
73	301	87·0	176	60	1·75	44·0	66·0	+31·8%
73A	400	115·8	172·0	49·0	1·58	73·2	60·8	+90·4%
74	289	83·7	155	48	1·45	57·7	51·5	+50·0%
74A	298	86·3	159·5	47·75	1·47	58·7	57·3	+50·6%
75	362	104·8	172·6	59·5	1·72	60·9	60·0	+39·3%
76	301	87·0	163	47·0	1·5	58·0	56·0	+55·3%
77	309	88·6	154·1	40·0	1·33	66·6	50·4	+75·7%
77A	—	—	163·2	53·0	1·55	—	—	—
78	321	92·6	171·2	50·0	1·56	59·3	65·0	+27·0%
78A	300	86·9	157·4	40·0	1·35	64·3	51·1	+70·0%
79	285	82·5	170·5	62·0	1·74	47·4	62·0	+50·3%
79A	338	97·8	172·3	55·0	1·65	59·2	56·0	+74·6%
80	3[illegible]2	93·2	169·8	52·5	1·60	58·2	55·6	+90·6%
81	369	106·6	172·6	58·8	1·7	60·2	57·0	+70·5%
82	336	97·2	163·1	46·5	1·47	66·1	56·7	+71·0%
83	281	81·3	161·0	42·75	1·41	57·6	54·1	+50·9%
84	293	85·0	168·8	54·2	1·61	52·8	54·9	+54·0%

Cockatoo Creek, August, 1931.

TABLE 2.

Comparison between Values for Surface Area Derived from Du Bois' Nomogram, and by Direct Measurement using the Paper Strip Method of Takeya (Fukuoka Acta Medica, 22/6/1929).

Metab. No.	Record No. of Native.	Tribe.	Surface Area Measured.	Surface Area Calculated.
64	16	Aruntа	1·73	1·75
81	17	Ilpirra	1·60	1·70
79A	20	Ilpirra	1·65	1·64
71	21	Ilpirra	1·66	1·67
77A	23	Ilpirra	1·55	1·55
83	27	Ilpirra	1·41	1·40

Cockatoo Creek, August, 1931.

TABLE 3.

Relationship between Sitting Height and Stature, and between Sitting Height and Weight.

No.	S.H. in centimeters.	S.H.$-\frac{H}{2}$	Pelidisi $\frac{\sqrt[3]{W} \times 10}{S.H.} = 100\%$.
64A	87·6	+3·3	98·37
65A	83·1	+2·2	103·4
66A	85·1	−1·4	100·7
67A	81·3	−0·2	93·1
68A	85·4	−0·1	98·8
69A	85·4	−0·1	95·6
70A	83·4	−2·7	97·6
71A	82·7	−3·0	98·6
72A	83·7	−0·3	99·8
73A	82·5	−3·5	95·5
74A	77·1	−2·6	101·4
75A	—	—	—
76A	—	—	—
77A	—	—	—
78A	77·5	−1·2	95·0
79A	84·1	−2·0	97·4
64	82·3	−0·8	100·5
65	—	—	—
66	88·6	+2·6	92·7
67	84·4	−1·9	99·5
68	78·3	−1·2	101·7
69	—	—	—
70	—	—	—
71	84·7	0·0	98·3
72	73·0	−8·3	103·5
73	83·2	−4·8	101·3
74	75·1	−2·4	90·7
75	84·2	−2·1	99·8
76	79·0	−2·5	98·4
77	76·0	−1·0	95·6
78	82·0	−3·6	96·7
79	83·7	−1·5	101·8
80	82·6	−1·3	97·6
81	85·3	−1·0	90·8
82	81·6	+0·1	94·9
83	82·1	+1·6	91·7

S.H. = Sitting height.
H = Stature.
W = Weight in grams.

Cockatoo Creek, August, 1931.

TABLE 4.

Exp. No.	Mouth Temp.	Air Temp. W.	Air Temp. D.	Skin Temp.	Pulse.	Resp.	Kata Thermometer. Wet.	Kata Thermometer. Dry.	Diurnal Variations. °C. Max.	Diurnal Variations. °C. Min.
64	35·9	0·0	0·0	26	58	13	18·4	11·2	29·19	—0·55
64A	35·6	0·0	0·0		62	19	,,	,,	,,	,,
65	35·55	—	10·0	27	61	10	—	—	,,	,,
65A	35·4	—	1·0		53	18	—	—	,,	,,
66	36·2	0·0	1·1	26	70	20	—	—	31·1	—0·87
66A	35·55	0·0	1·1		57	24	—	—	,,	,,

TABLE 4 (continued).

Exp. No.	Mouth Temp.	Air Temp. W.	Air Temp. D.	Skin Temp.	Pulse.	Resp.	Kata Thermometer. Wet.	Kata Thermometer. Dry.	Diurnal Variations. °C. Max.	Diurnal Variations. °C. Min.
67	36·2	8·33	13·89	30	68	18	19·4	22·0	31·1	—0·87
67A	35·1	7·78	10·0		62	21	—	—	„	„
68	35·1	7·78	10·0	27	60	10	—	—	33·0	6·7
68A	36·6	7·78	10·0		62	20	—	—	„	„
69	37·4	7·78	10·0	29·5	80	24	12·25	15·5	„	„
69A	35·7	8·33	13·9		58	18	—	—	„	„
70A	35·1	4·4	6·6		54	11	13·8	16·7	30·1	4·2
71	36·5	—	—	33·0	54	20				
71A	37·0	4·4	6·6		66	17	13·8	16·7		
72	35·0	—	—	23·3	40	18				
72A	35·1	4·4	6·6		64	15	13·8	16·7		
73	37·1	8·33	12·2	28·0	52	16			28·3	3·3
73A	34·5	4·4	8·8		57	17				
74	36·0	4·4	6·6	27·0	69	12				
74A	35·6	5·5	8·9		66	15			26·67	2·78
75	34·5	6·4	9·6	28·0	72	14				
76	33·6	5·5	8·9	28·1	60	15				
77	36·0	5·5	8·9	27·0	82	15				
78	31·0	—2·2	0·0	23·0	63	14			29·4	—2·2
78A	—	—2·2	0·0	—	—	—				
79	34·2	—2·2	0·0	25·4	54	16				
79A	34·5	+2·2	6·0	—	66	19	16·8	13·6		
80	36·0	+2·2	6·0	27·4	80	18				
81	33·8	—0·5	1·67	—	98	14			30·7	—0·3
82	35·2	2·8	6·7	—	72	15				
83	35·2	5·5	6·1	—	62	17				
84	33·0	9·4	10·5	—	44	11	51·0	23·6		

Cockatoo Creek, August, 1931.

TABLE 5.

Blood Pressure—Supine Position.

Age and Sex.		Mm. Hg. Systolic.	Mm. Hg. Diastolic.	Age and Sex.		Mm. Hg. Systolic.	Mm. Hg. Diastolic.
19	F.	91	68	45	F.	127	82
30	M.	110	75	40	M.	130	92
25	M.	114	78	35	F.	110	73
40	M.	100	71	18	F.	102	68
35	M.	108	74	30	M.	112	77
20	F.	100	60	15	M.	92	56
14	F.	90	56	25	M	101	70
45	F.	99	62	60	M	128	83
30	M.	115	74	60	M.	120	86
40	M.	120	78	60	F.	110	73
28	M.	96	59	40	M.	111	73
35	M.	121	79	19	F.	102	74
29	M.	122	78	17	M.	102	69
35	F.	92	56	17	M.	101	74
60	M.	102	64	17	M.	91	57
30	M.	101	72	25	M.	98	70
22	M.	101	68	20	M.	100	59
60	M.	124	87	19	M.	95	58
23	M.	112	72	50	M.	109	77
18	M.	98	71	60	M.	126	89
60	M.	120	86	35	F.	106	58
50	M.	112	72	68	M.	119	88
55	M.	118	74	28	M.	91	57
33	M.	110	75				

OBSERVATIONS ON THE PROTEINS OF OX SERUM

by

HENRY FRANCIS HOLDEN AND MAVIS FREEMAN

(From the Walter and Eliza Hall Institute, Melbourne).

(Submitted for publication 10th March, 1933.)

Of the methods of separation of serum proteins, ammonium sulphate in increasing concentrations followed by dialysis is the chief one in use to-day. Various tests have been employed to ascertain the individuality of the products obtained. Frequent use has been made of the polarimeter, following the work of Hopkins (1900) on egg albumin and of Young (1922) on that protein and on crystalline horse serum albumin.

In 1927 the validity of determinations of the specific rotation of proteins for their characterization was examined and adversely criticized by Hewitt (1927), who found that the optical activity of a protein was influenced by the presence of small amounts of material removable by extraction with alcohol and ether under appropriate conditions without otherwise impairing the protein so treated.

Hewitt described a series of experiments in which proteins were precipitated from aqueous solutions at temperatures below 0°C. with alcohol and ether, and subsequently extracted with boiling ether in a Soxhlet apparatus. The rotations of these proteins were all higher than the values given in the literature. Corresponding proteins of different species of animals had identical optical activities.

In a previous paper (1930) it was pointed out that the amount of lipoid material in crystalline egg albumin was extremely small, and other explanations of Hewitt's findings were suggested. In the present paper are collected the results of experiments on the proteins of ox serum. The proteins of ox serum were prepared by methods based on the use of inorganic reagents only. Variations were observed in the optical activity of these proteins which lay outside any experimental error, and which were not related to the variations in the methods of preparation.

These experiments confirm the views expressed by Svedberg (1928) that prolonged treatment of a protein by any of the methods generally adopted does not result in any further purification. On the contrary, much of the material is often changed into substances that differ from the parent protein in molecular weight but that so resemble it in many respects that they cannot readily be removed.

Sörensen (1923) prepared many specimens of serum globulins, and deter-

mined their solubilities in solutions of neutral salts. He used elaborate methods of preparation, involving the use of many fractional precipitations and repeated dialyses at 0°C. in the presence of toluene, in an effort to obtain complete separation. He confirmed and explained the finding of Mellanby (1905) that the solubility of crude euglobulin in salt solutions depends on the amount of excess solid protein present as well as on the concentration of the salt. He concluded that all specimens of these proteins are mixtures of the two of inconstant composition, that pure pseudoglobin contains no phosphorus, and that the purest known specimens of either protein are capable of further disintegration, yielding some of the other. His experiments on ovalbumin show that the presence, or absence, of phosphorus in some proteins has little effect on their determinable physical characters. As will be seen, the experiments described below support some of his views, while offering other explanations of others. Chick (1914), on the other hand, suggested that euglobulin was produced from pseudoglobulin by a process of denaturation, and owed its solubility in salt solutions to its lipoid content.

EXPERIMENTAL.

An attempt was first made to prepare ox serum euglobulin with a constant rotation. Ox serum was diluted with varying volumes of water, and neutral saturated ammonium sulphate solution added in quantities sufficient to make the mixture 33%, 40%, or 50% saturated. The precipitated protein was filtered off, reprecipitated once, twice, or thrice, depending on the experiment, and its solution dialysed. The precipitated euglobulin was suspended in water, the minimum amount of sodium chloride requisite for solution added, and the protein reprecipitated by dialysis. This was done several times. In other experiments no ammonium sulphate was used, the euglobulin fraction being precipitated from serum by dialysis.

After a few precipitations by dialysis, only a trace of protein soluble in water was removable by further treatment, but the solution of euglobulin so obtained, which was after the first precipitation perfectly bright, becomes progressively more opaque. Again, instead of it being necessary for solution of the protein, as at first, to add only a few c.c. of 1% sodium chloride solution to 100 c.c. of a 2–3% suspension of euglobulin, it became necessary, after several precipitations by dialysis, to make the concentration of sodium chloride in the protein solution up to some four or five per cent. The euglobulin was generally dissolved for polarimetry in very dilute ammonia, which yielded a clearer solution. The rotations were determined for the green mercury line. The pH of the solutions was measured electrically. The concentration of the protein was determined by coagulation in hot ammonium sulphate solution, washing, drying, and weighing the coagulum. In the case of some of the serum proteins which were examined in salt-free solution, a known volume of the solution was evaporated to dryness, and the residue weighed. A comparison of the two methods showed that the values obtained agreed. As Table 1 shows, in spite of all precautions the specific rotation of ox serum euglobulin was found to be a variable quantity. It did not appear to depend on the method of preparation employed, or upon the season of the year. Variations within the physiological range of the pH of the final solution were of small effect. On the acid side of its isoelectric point the protein was more strongly laevo-rotatory.

A similar technique was attempted in the case of ox serum pseudoglobulin. The name of this water-soluble protein, precipitated by half saturation of its solution with ammonium sulphate, is most unfortunate, but it has persisted for a considerable period, and any attempt at reform would merely confuse. It has the properties of an albumin, being soluble in water,

and, like some of the vegetable albumins, insoluble in half-saturated ammonium sulphate solution. Specimens of pseudoglobulin used in this work were prepared by dialysis of the mixed globulins precipitated by half saturation with ammonium sulphate from the serum diluted with several volumes of water. Other specimens were prepared from the first precipitate of euglobulin obtained by dialysis of a solution of the mixed globulins. This precipitate contained a large proportion of pseudoglobulin, and, on solution in dilute sodium chloride solution and dialysis, much of it remained in solution. Specimens were also prepared from sera by removing the bulk of the euglobulin by one-third saturation with ammonium sulphate, followed by half saturation, and dialysis.

TABLE 1.

Ox Serum Euglobulin.

Method of preparation.	Solvent	PH	[α] 5461
$(NH_4)_2SO_4$ and dialysis	NaCl	6·3	—59·8
"	"	5·6	—55·0
"	"	6·6	—61·0
"	"	6·4	—56·0
"	NH_4OH	8·2	—55·6
Repeated dialysis	NH_4OH	—	—54·2
"	NH_4OH	8·0	—51·5
	HCl	3·2	—66·9
"	NH_4OH	8·5	—56·3
	HCl	3·4	—64·0
	HCl	2·3	—84·5
Dialysis and Hardy precipitation	NH_4OH	8·5	—66·0

The values in brackets are from one preparation observed under various conditions.
The rotations actually observed were from 1·0° to 2·1°, giving a probable error of about ±0·6°.

Two factors rendered the preparation of pseudoglobulin difficult. The first was its instability. Even so mild a treatment as precipitation at 0°C. by ammonium sulphate, followed by filtration and removal of salts from the precipitate by dialysis in a pyroxylin membrane, changed a portion into a protein insoluble in water. The second factor was the tendency of pseudoglobulin to dissolve other proteins, particularly euglobulin.

After removal of salts by dialysis, a pseudoglobulin solution containing some four or five per cent. of this protein was able to dissolve a large amount of euglobulin at the isoelectric point of the latter. On dilution of the solution to a pseudoglobulin concentration of not more than 2%, a large amount of the impurity was precipitated by careful adjustment of the reaction. Though no further precipitate of euglobulin occurred on further dilution, it was quite impossible to feel any confidence that none remained in solution. The precipitation zone is very narrow, and it was difficult to ensure complete precipitation. The instability of pseudoglobulin precluded any subsequent concentration by the methods ordinarily used in such cases.

Table 2 shows that, in spite of every precaution, the optical activity of the purified pseudoglobulin varied outside the limits of error of the methods used for its determination. A careful examination was made of the solutions used for polarimetry, and any showing more than a trace of euglobulin were discarded. The addition of small amounts of euglobulin should scarcely affect the apparent specific rotation of pseudoglobulin, since their rotatory powers are much the same.

TABLE 2.

Ox Serum Pseudoglobulin.

pH	[α] 5461	pH	[α] 5461	pH	[α] 5461
5·7	—52·9	5·0	—56·9	6·0	—59·8
6·0	—53·1	5·7	—57·1	3·1	—60·0
5·5	—53·2	5·7	—57·2	6·2	—60·2
6·1	—53·2	5·8	—57·2	9·6	—60·4
6·2	—53·7	6·4	—57·3	6·0	—60·4
5·0	—53·8	4·9	—57·5	5·3	—60·9
7·0	—54·0	6·1	—57·6	6·4	—60·9
5·6	—54·1	6·5	—57·9		
		5·2	—58·0	5·9	62·0
				4·5	62·2
6·3	—54·9	7·5	—58·3	6·0	62·3
5·9	—55·1	6·0	—58·5	5·8	62·9
6·6	—55·2	9·6	—59·0	2·7	74·4
5·5	—55·8				
6·9	—56·4				

The rotations observed varied from 0·9° to 2·7°. The probable error is about ±0·6° for most of the values.

Most of the determinations fall into 3 groups covering about 1° each.

A review of the experimental procedure adopted does not, however, suggest any cause for this.

In the first experiments on ox serum albumin the serum was obtained from defibrinated blood. In ox serum is a yellow pigment associated with the albumin fraction which interferes with the polarimetric determination. Many attempts were made to remove it, without success. It was found best to remove the globulins by the addition of an equal volume of saturated ammonium sulphate solution to the undiluted serum. After filtration the albumin was precipitated from the filtrate by the addition of sufficient ammonium sulphate, filtered off, and its solution dialysed. The whole procedure was carried out as rapidly as possible in a refrigerator below 2°C. The results showed less variation than those for either of the globulins, but, as will be seen, this is not considered to indicate that the protein was particularly pure. Other methods employed for the precipitation of the albumin were the addition of the minimum requisite quantity of acetic acid to the solution in half-saturated ammonium sulphate, and warming the half-saturated ammonium sulphate solution. In either case a little of the albumin was converted into a meta-protein, a precipitate of which could be filtered off from the resultant albumin solution after dialysis at its isoelectric point. The specific rotation of the albumin finally obtained was higher than that of the specimen prepared from the same batch of serum by the use of ammonium sulphate alone. If for serum was substituted oxalated plasma, the specific rotation of the albumin, obtained by precisely the same technique as used for the albumin from serum, was a few degrees higher and the solution was less coloured.

TABLE 3.

Albumin prepared from Ox Serum from Defibrinated Blood.

(a) By saturation with ammonium sulphate, without subsequent dialysis.

pH	[α] 5461
5·8	—62·6
6·1	—63·0

TABLE 3 (continued).

Albumin prepared from Ox Serum from Defibrinated Blood.

(b) Same, but ammonium sulphate removed by dialysis.

pH	[α] 5461
6·6	—62·1
6·0	—62·5
6·6	—62·6
6·1	—63·0
6·0	—63·4
6·3	—64·1
6·8	—64·4
6·6	—64·6
5·9	—65·0
5·7	—65·0

(c) By the addition of acid to the solution half-saturated with ammonium sulphate.

5·9	—69·1
5·6	—68·4

(d) By warming the solution half-saturated with ammonium sulphate.

6·3	—65·7
7·0	—67·8

(e) By precipitation from the dialysed solution by Hardy's method, but without drying.

—	—64·0

The high rotations in (c) and (d) are probably due to the presence of some denatured albumin dissolved by the native albumin.

The rotations observed varied from 1·3° to 2·0°, giving a probable error of ±0·5°.

In some experiments the fibrinogen was coagulated by the addition of calcium chloride and the fibrin brought out in the form of a coarse flocculum by stirring with a glass rod. The ammonium sulphate solution was then added to the serum as in the other experiments. The modification yielded an albumin of about the same specific rotation as when the fibrinogen was precipitated with the globulins.

TABLE 4.

Albumin prepared from Ox Plasma by Saturation with Ammonium Sulphate and subsequent Dialysis.

(a) Fibrinogen removed with the globulin fraction.

pH	[α] 5461
6·7	—65·2
6·6	—66·0
5·9	—68·7
6·7	—69·2
6·7	—69·9

(b) Fibrinogen coagulated before globulin fraction removed.

6·7	—67·0
6·7	—67·6
6·9	—70·6
6·4	—69·4

The rotations observed varied from 2·5° to 4·8°, giving a probable error of ±0·3°.

If the albumin were precipitated with ethyl alcohol at a low temperature and redissolved rapidly without allowing it to dry, the specimen, while clear and apparently soluble in water, had a specific rotation a little higher than that of one prepared by the use of ammonium sulphate only.

THE EFFECT OF ORGANIC SOLVENTS ON PROTEINS.

Hewitt (1927) extracted the mixed proteins of serum, by the method of Hardy, with alcohol and ether at —5°C. before separating them with ammonium sulphate. It has long been known that many forms of chemical treatment alter the proportions of the relative fractions of serum protein separated by different concentrations of ammonium sulphate in the direction of an increase in the amounts of the more precipitable fractions and a decrease in the serum albumin. It seemed reasonable, therefore, to examine the effect of the Hardy precipitation on the three main serum proteins separately. Every precaution was taken to keep the temperature as low as possible, and the whole experiment was performed in a refrigerated chamber. It was found uniformly that, though the mixed proteins of ox serum may be so treated, dried, stored, and redissolved, the protein fractions separated by ammonium sulphate and dialysis may not.

A large proportion of either of the globulins is converted to a meta-protein, and, while the albumin may be redissolved in water, provided that that process is carried out rapidly and the protein not permitted to dry, it becomes entirely insoluble if, at below 0°C., it is washed free from water with the anhydrous alcohol-ether mixture and the organic solvents removed in a vacuum at a low temperature according to the method which is suitable for the mixed serum proteins. Polarimetric determinations on the portions of the proteins that remained soluble in water or salt solutions showed that they possessed rotations higher than those of the proteins untreated with organic solvents, though not so high as some of the values found by Hewitt. It is, however, doubtful if these increases are due either to the removal of lipoids or to the greater freedom of these proteins from each other or from the products of their partial disintegration. The mixed proteins of some fresh rabbit serum had a specific rotation of —56·4° for the yellow mercury line. After precipitation by Hardy's method and drying this fell to —52·8°. After storing in the dry state for six months at room temperature it had risen to —58·7°. If the effects observed by Hewitt were due to the simple removal of lipoids, an immediate rise in the rotation of the mixed proteins should also have been observed.

THE MUTUAL SOLUBILITY OF PROTEINS.

Hardy (1905) observed that fresh serum has the power of dissolving a large quantity of the precipitate of crude euglobin obtained by dialysis of serum. In the course of the present work it was observed that, during the pressure dialysis of the mixed globulins precipitated from serum by half saturation with ammo-

nium sulphate, the euglobulin was precipitated, not as a white amorphous powder, but as a clear gel at the bottom of the membrane. After decantation of the supernatant fluid containing most of the pseudoglobulin, the gel was stirred with water. It disintegrated, yielding a white precipitate of euglobulin, while pseudoglobulin was found in the supernatant fluid.

The precipitate of euglobulin was dissolved in sodium chloride solution, redialysed, and the extraction with water repeated. After a few repetitions the gel formation did not occur; the euglobulin required a much higher concentration of salt (up to 5%) for solution, and the solution was nearly opaque.

If a solution containing some 4% or more pseudoglobulin were adjusted to pH 5·8 and dialysed till no more euglobulin was precipitated, a further heavy precipitate was obtained on dilution of the solution to a concentration of about 1·5%–2%. Little euglobulin could be obtained by further dilution. The euglobulin precipitated on dilution appeared first as a white amorphous powder, which soon changed on standing to the gel form referred to above, with absorption of pseudoglobulin. These gels, like the first precipitate obtained by the dialysis of serum, required for solution only a very small amount of sodium chloride. As Sörensen showed, the ease with which euglobulin dissolves at its isoelectric point in salt solutions is mainly dependent on the very variable amount of pseudoglobulin (or other protein) associated with it.

The albumin solutions prepared during the earlier stages of this work frequently, after the removal of salts by dialysis, yielded on dilution a small amount of a precipitate, which on centrifuging was obtained as a gel at the bottom of the cup. This gel was readily soluble in dilute salt solutions. Several workers have observed that treatment of serum by warming with very dilute acid or alkali for brief periods does not yield a precipitate on neutralization, but that the proportion of protein precipitable by half saturation with ammonium sulphate is thereby increased. This phenomenon has been described as a conversion, *in vitro*, of albumin into globulin, and attempts have been made to link it with the increased globulin content of immune sera.

Moll (1904), in a series of experiments on various preparations of serum albumin, found it apparently possible to convert some of this protein into euglobulin. He warmed for brief periods solutions of serum albumin containing small amounts of alkali, and endeavoured to estimate the amounts of euglobulin and pseudoglobulin produced by this means. While his careful observations can be repeated, it would appear that he did not realize the extreme difficulty of separating quantitatively the small amounts of proteins with which he dealt.

Some experiments on similar lines were made, using acid in place of alkali. It was first observed that if pseudoglobulin, carefully freed from euglobulin, were treated for a few hours at room temperature with very dilute acid (pH 2·5–3) much of it was apparently converted into euglobulin. The unsatisfactory methods available for the separation of these proteins must be remembered in judging such an experiment, but at least after such treatment it was possible to separate from a pseudoglobulin solution a large amount of euglobulin by dilution at the isoelectric point, whereas previously a sample treated in this manner yielded little or no precipitate. If the remainder of the pseudoglobulin were carefully concentrated by ultrafiltration the process could be repeated.

Compared with the serum globulins, ox serum albumin was relatively resistant to the action of acids. The former are completely denatured at pH 2 in a few hours at 40°C., and in a few minutes at 60°C., while under these conditions serum albumin suffers very little apparent effect. If the heating of a solution of serum albumin were more prolonged, and the reaction of the fluid subsequently adjusted to pH 4·8, a small amount of precipitate was thrown down and could be centrifuged off. The liquid yielded a heavy precipitate on the addition of an equal volume of a saturated solution of ammonium sulphate. In these experiments this precipitate was filtered off, dissolved in water, in which it formed a somewhat opalescent solution, and the solution dialysed. When the salt was removed a precipitate occurred in the form of an opalescent gel at the bottom of the membrane. This gel was soluble in sodium chloride solution. On solution and further dialysis the protein disintegrated into serum albumin and an insoluble meta-protein. Similar effects were obtained by the use of alcohol for a brief time at room temperature. The albumins of sheep and horse sera also showed the effect.

Another experiment may be described which appears to have a similar explanation. Some serum albumin was prepared, and completely denatured by prolonged treatment with hydrochloric acid at 37°C. By repeated precipitations at its isoelectric point its freedom from all native albumin was assured. It was quite insoluble either in water or salt solutions at this point, though freely soluble in either dilute acid or dilute alkali. A 2% solution in dilute sodium carbonate was made, and 1 c.c. added to 2 c.c. of a 3% native serum albumin solution. On adjusting the reaction to pH 4·8 (the isoelectric point) a bulky precipitate was thrown down and centrifuged off. This precipitate was readily soluble in dilute acetic acid, sodium carbonate, or sodium chloride. Pseudoglobulin was able to replace the native albumin, and denatured egg albumin, or casein, the denatured albumin.

It was found impossible to dissolve denatured ox globin by this method. It had previously been found that small amounts of salts seriously decrease the solubility of this protein in both acids and alkalis. Native egg albumin was quite unable to dissolve any insoluble protein tried in the presence of neutral salts.

Van Slyke and Hart (1905) succeeded in preparing casein in a form which was insoluble in water but soluble in warm 5% sodium chloride solution, by washing the precipitate of casein obtained from skim milk by the addition of hydrochloric acid with several changes of water. The work was done in 1905, before precise methods of measuring concentrations of hydrogen ions were available. Provided that the acidity of the solution be adjusted to pH 4·6, the isoelectric point of casein, there seems no doubt that casein is quite insoluble in any concentration of sodium chloride or other salt. If, however, a solution of casein containing 1–2% be made in the minimal strength of sodium hydroxide, and dilute acetic acid be added carefully, allowing the precipitate first formed to redissolve before more acid is added, some casein is precipitated at pH 5·4 and does not redissolve. As more acid is added more casein is precipitated, and the solution gradually becomes more acid. If the addition of acid ceases when pH 5·1 is reached (or earlier), and the precipitate centrifuged off, that precipitate, while quite insoluble in water, is readily soluble in dilute sodium or potassium chloride solution. If the addition of the acid is continued till pH 4·6 is reached the whole of the casein is precipitated, and none dissolves in the salt solution.

DISCUSSION.

The variations, recorded in this paper, in the specific rotations of the proteins of ox serum serve to indicate that they are probably not individual proteins, but variable and unstable mixtures. This is borne out by the facts that, as Sörensen showed, any attempt at purification of pseudoglobulin results in the appearance of a small quantity of a protein with the solubility of euglobulin, and that the former protein may be extracted from a preparation of the latter, with concomitant changes in the appearance of its solution and degree of solubility in salt solutions.

The variations observed in the rotation of ox serum albumin, when carefully prepared from ox plasma, are smaller than those in the rotations of the globulins. There is, however, no ground for considering it to be other than an inconstant mixture. The fact that the two well-known crystallizable proteins, ovalbumin and horse serum albumin, show constant rotations under defined conditions strongly suggests that non-crystalline proteins of uncertain rotation are in reality mixtures. The experiments on the effects of alcohol on the rotation of the mixed proteins of rabbit serum and on the solubility of the "individual" proteins of ox serum indicate that the presence of lipoids is not the sole factor in determining the rotations of the serum proteins.

If it be recognized that in the presence of some native proteins small amounts of neutral salts cause a meta-protein to dissolve at its isoelectric point much of the difficulty in satisfactorily preparing some proteins will be understood. It would appear that pseudoglobulin in fairly high concentration can dissolve euglobulin even in the absence of salts. Oxyhaemoglobin and serum albumin in high concentrations can dissolve proteins of the meta-protein type which are quite insoluble in salt solutions. If erythrocytes be haemolyzed with the minimum amount of water, and the liquid centrifuged, the clear solution of haemoglobin so obtained contains in addition much dissolved stroma protein which is precipitated on further dilution. A mixture of native and denatured serum albumins precipitated at the isoelectric point resembles euglobulin in that it is soluble in dilute acids, alkalies, or salt solutions. The optical activity of such a mixture is, of course, quite irregular.

The production in a solution of pseudoglobulin of a protein with the properties of euglobulin, after previously freeing the former protein from all discernible traces of the latter, can apparently be readily accomplished by the brief action of dilute acids. It would seem doubtful, however, whether the reverse process can occur. Accepting as correct Sörensen's picture of complex compounds composed of varying amounts of euglobulin and pseudoglobulin, it would appear that a limit is approached at which no more pseudoglobulin molecules exist in the complex. At, or before, that stage it would appear probable that the distinctive salt-solubility of the euglobulin would disappear also. Until that stage is reached

continuous fractionation at suitable concentrations of salt would, as he shows, split the complexes into groups containing more and less pseudoglobulin, and correspondingly more, and less, soluble in dilute salt solutions. The exceedingly slight solubilities recorded in that paper, in some cases less than 0·01% of protein in dilute salt solutions, suggest strongly that, whether due to purification or the prolonged action of toluene, the number of pseudoglobulin molecules remaining in the complex is small. Since a native protein can, in the presence of salts, dissolve a denatured protein, any proof of the conversion of euglobulin into pseudoglobulin, as distinct from the breakdown of complexes into other complexes of differing solubilities in salt solutions, would necessitate the preparation of pure euglobulin, or at least the accurate determination of the relative amounts of the two proteins in a mixture of the two.

The so-called conversion *in vitro* of albumin to globulin is in fact the denaturation of a small portion of the serum albumin. The denatured portion is dissolved by the remaining native protein present and precipitated by ammonium sulphate partly in the euglobulin fraction and partly in the pseudoglobulin fraction. Into both fractions it carries some native albumin. The difficulty in quantitatively separating the denatured derivatives from a native protein is well shown in the case of globin. As saturated ammonium sulphate solution is added to a solution containing globin and denatured globin the latter is precipitated by degrees right up to half-saturation with the salt. In the absence of native globin a very small amount of ammonium sulphate precipitates the latter completely at any hydrogen ion concentration. In the presence of euglobulin it would appear doubtful if pseudoglobulin could be prepared free from serum albumin by salt precipitation, even if the former protein were not affected by the process.

In the case of the solution of casein by neutral salts it would appear that the one protein—if casein be an individual—plays the part both of the native and of the denatured protein. The precipitate of casein first formed has associated with it sufficient casein in solution to redissolve it when a neutral salt is added.

Finally it may perhaps be permitted to enquire whether serum euglobulins really are a distinct class of individual proteins allied by implication to the crystallizable globulins of the vegetable kingdom. Their sole resemblance is their solubility in salt solutions. They differ from the latter in not being crystalline, in their extreme instability towards organic solvents, in the opacity of their solutions, and the difficulty with which they pass through filters. In this paper it is shown that suitable artificial mixtures of native with denatured proteins may exhibit in considerable degree the solubility behaviour of euglobulins, while polarimetric evidence is produced that euglobulins, as ordinarily prepared at least, are variable mixtures.

While the presence of lipoid material does not appear essential to the salt-solubility of proteins, the suggestion of Chick (1914) concerning the formation of euglobulin from pseudoglobulin would appear to be substantially correct.

SUMMARY.

The variations in optical activity of the proteins of ox serum are examined. The rotations of the proteins are not constant. Experiments are described on the supposed *in vitro* conversion of serum albumin to serum globulin, the solubility of casein, and on the solubility of a meta-protein by a native protein.

It is suggested that serum euglobulin may be a mixture of pseudoglobulin with a meta-protein. Solutions of serum euglobulins resemble those of meta-proteins in the great difficulty with which they can be filtered and in their behaviour in the vicinity of their isoelectric points towards small amounts of electrolytes. The physical chemistry of their solutions marks them off from the albumins and pseudoglobulins. There is something to be said for the hypothesis that they are intimate mixtures of pseudoglobulin with a meta-protein, not separable by present methods. It is possible that they contain in addition some serum albumin.

REFERENCES.

Chick, H. (1914): Biochem. J., 8, p. 404.
Hardy, W. B. (1905): J. Physiol., 33, p. 251.
Hewitt, L. F. (1927): Biochem. J., 21, p. 216.
Holden, H. F. and Freeman, M. (1930): Austral. J. exp. Biol., 7, p. 13.
Hopkins, F. G. (1900): J. Physiol., 25, p. 306.
Mellanby, J. (1905): J. Physiol., 33, p. 338.
Moll, L. (1904): Beit. chem. Physiol., 4, p. 563.
Sörensen, S. P. L. (1923): C.R. Lab. Carlsberg, 15, No. 11.
Svedberg, T. and Sjögren, B. (1928): J. Amer. Chem. Soc., 50, p. 3318.
Van Slyke, L. L. and Hart, E. B. (1905): Amer. Chem. J., 33, p. 469.
Young, E. G. (1922): Proc. Roy. Soc., 93B, p. 15.

THE THRESHOLD FOR THE RENAL EXCRETION OF INORGANIC PHOSPHATES IN THE SHEEP

by

RODGER H. WATSON[1]

(From the Division of Animal Nutrition of the Commonwealth Council for Scientific and Industrial Research at the University of Adelaide, South Australia).

(Submitted for publication 27th March, 1933.)

Addis, Meyers, and Bayer (1925), Brain, Kay. and Marshall (1928), Walker (1932), and others have shown that in the rabbit and in man a relation exists between the rate of excretion of inorganic phosphate in the urine and its concentration in the blood. Brain, Kay, and Marshall (1928), and Walker (1932), by extrapolation from their observations, have concluded that in man a threshold exists at 2–3 mgs. P/100 c.c. blood below which excretion of inorganic phosphate ceases, or, at least, is at a minimum.

In the sheep, and, as far as I can gather, in herbivora generally, usually but a trace of phosphorus is excreted in the urine, and inorganic phosphate is indetectable. Some sheep, receiving an addition to the normal diet of 1 gm. of phosphorus as calcium phosphate, whose urine I had the opportunity to examine, were, however, excreting urine containing 10–60 mgs. P/100 c.c. (fig. 1 and Table 1). Also, from Weiske's (1872) observations on a goat fed on milk, and Gouin and Andouard's (1907) on the calf, it would appear that, when living on diets of animal matter containing more assimilable phosphorus, the herbivora may excrete it through the kidneys.

Forbes and Keith (1914), in a review on the literature on phosphorus compounds in animal metabolism, mention that during starvation considerable amounts of phosphorus appear in herbivorous urine, and Marston (unpublished observations) found that after sheep were fasted 2–3 days phosphate began to be excreted by this route, and the excretion rose to more than 1 gm. phosphorus per day. The amount of inorganic phosphate in the blood of these sheep was not determined, but, as Cavins (1924) and Wilder (1929) discovered in rachitic rats, I find, from experiments to be detailed below, that a rise in concentration of inorganic phosphate in the blood also occurs in sheep during fasting.

---

[1] Robert Philp Scholar of the University of Queensland.

These various observations suggest that a threshold for inorganic phosphate also exists in the herbivora, particularly in the sheep.

The excretion of phosphate in the urine may, however, be conditioned by the acid-base equilibrium in the body. The sheep, during starvation, changes from its potentially basic vegetable diet to the potentially acid diet of its own tissues.

METHODS.

Collection of Urine.

The animals used throughout were ewes. The urine was collected by placing the animal in a metabolism cage, with provision for collecting urine and faeces separately, and in addition catheterizing before and after the period of collection. With short periods of about two hours micturition rarely took place, so that all the urine secreted was obtained with the catheter.

Collection of Blood.

Blood was drawn from the jugular vein as soon as possible after the sheep had been catheterized. Where serum was used the blood was collected under paraffin, for observations of Maizels and Hampson (1927) and Marston (unpublished) indicate that a migration of (HPO_4), as well as Cl, takes place between the corpuscles and the serum if the pH varies.

Estimation of Total and Inorganic Phosphorus.

The method of Fiske and Subbarow (1925) was used. Blood was deproteinized with trichloracetic acid. For total phosphorus determinations the urine was dry-ashed with magnesium nitrate at low red heat. Inorganic phosphorus determinations could only be made on the urine if more than 5 mgs. and satisfactorily only if about 15 mgs. P/100 c.c. were present. In the latter case the determination was done directly, but where less was present the urine was deproteinized with trichloracetic acid.

Determination of pH.

This was done either colorimetrically by means of the Hellige comparator or with the quinhydrone electrode.

EXPERIMENTS.

The Renal Excretion of Phosphorus in the Urine of Sheep, the Diet of which contained Various Quantities of Phosphorus.[2]

A few observations were made on pen-fed sheep, some of which happened to be receiving a daily ration of only 0·5 gm. phosphorus, and others, in addition,

[2] I am indebted to Mr. A. W. Peirce for allowing me to use these sheep, and for his help in making the observations.

1 gm. of calcium phosphate. The results of some of these observations are presented in fig. 1. The bladder was emptied by catheterization and blood collected. After an interval of an hour the urine secreted over the period was collected with the catheter and a second sample of blood taken. The mean of the concentrations of inorganic phosphate in the two samples of blood was taken as being representative of the concentration throughout the period. The rate of excretion of phosphorus was derived from the amount of urine secreted per hour and the concentration of phosphorus in it.

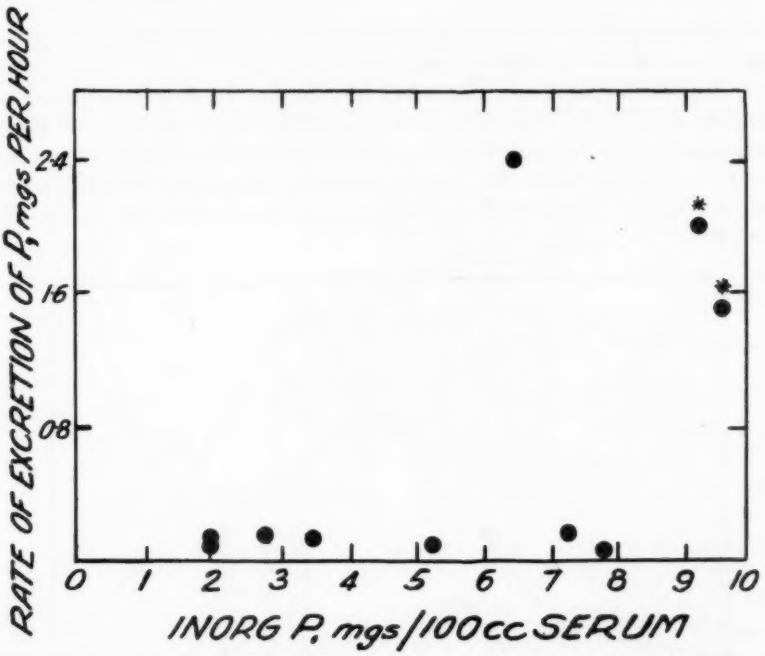

Fig. 1. The relation of the renal excretion of phosphorus to the inorganic phosphorus of the serum. Sheep receiving $CaHPO_4$.

* These are minimum values, for only 4·4 and 10 c.c. of urine were obtained in the hour, so that the sheep had probably urinated in between.

In a few sheep, only the concentration of inorganic phosphate in the urine was determined (Table 1). Figures for the concentration of inorganic phosphate in the blood, from determinations made ten days before and ten days after the observations on the urine, were, however, available. All these sheep were receiving 1 gm. of calcium phosphate in addition to that in their diet.

TABLE 1.

Inorganic Phosphate in the Urine and the Blood of Sheep receiving $CaHPO_4$ in the Diet.

Concentration of Inorganic Phosphate in Urine. mgs. P/100 c.c.	Concentration of Inorganic Phosphate in Blood. mgs. P/100 c.c.
Tr.	5·0, 5·8
19·7	5·4, 5·9
3·9	7·7, 7·5
29·1	7·5, 8·3
57·3	8·3, 8·6

Higher concentrations of inorganic phosphate in the blood were accompanied by the appearance of inorganic phosphate in the urine.

It requires a period of a few weeks to raise the concentration of inorganic phosphate in the blood sufficiently by increasing the amount in the diet. Fasting, as mentioned in the introduction, brings about the excretion of phosphate in the urine in 2–3 days. It has thus been employed to study the relationship of the renal excretion of phosphate to its concentration in the blood and to the pH of the urine.

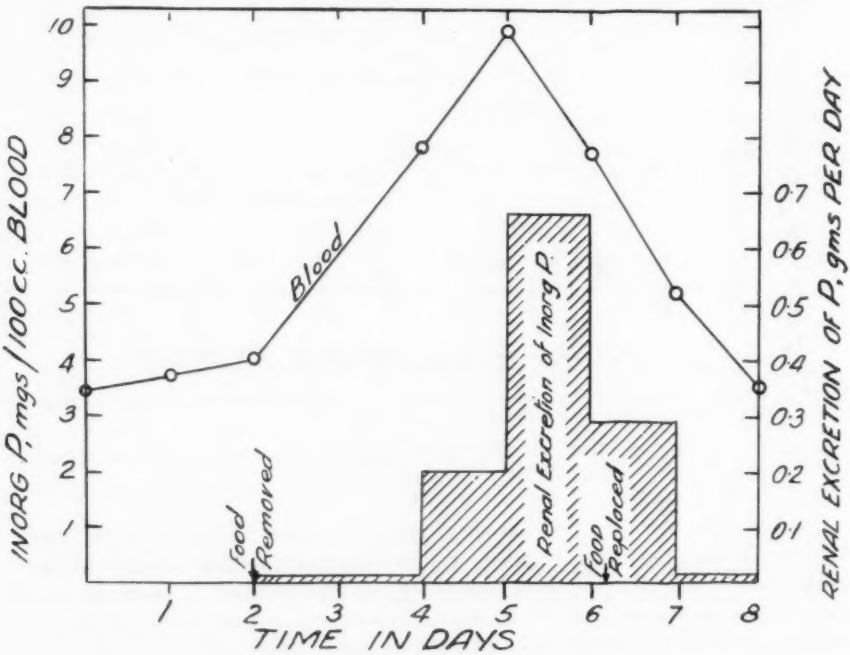

Fig. 2. The inorganic phosphate of the blood and the renal excretion of inorganic phosphate during starvation. Sheep W_3–96.

The Inorganic Phosphate of the Urine and the Blood and the pH of the Urine during Starvation.

Simultaneous estimations were made of the concentration of inorganic phosphate in the blood and the rate of excretion of inorganic phosphate in the urine of fasting sheep. The data of a typical experiment is plotted in fig. 2.

The inorganic phosphate of the blood rose during inanition. This was shortly followed by an excretion of phosphates in the urine. On resumption of feeding the concentration of inorganic phosphate in the blood fell, and phosphates almost entirely disappeared from the urine.

The observations on W_3–96 were later repeated at more frequent intervals. A complete series of observations was not obtained, but it was noted that excretion of inorganic phosphate in the urine began when the concentration of inorganic phosphate in the blood rose to about 8 mgs. P/100 c.c.

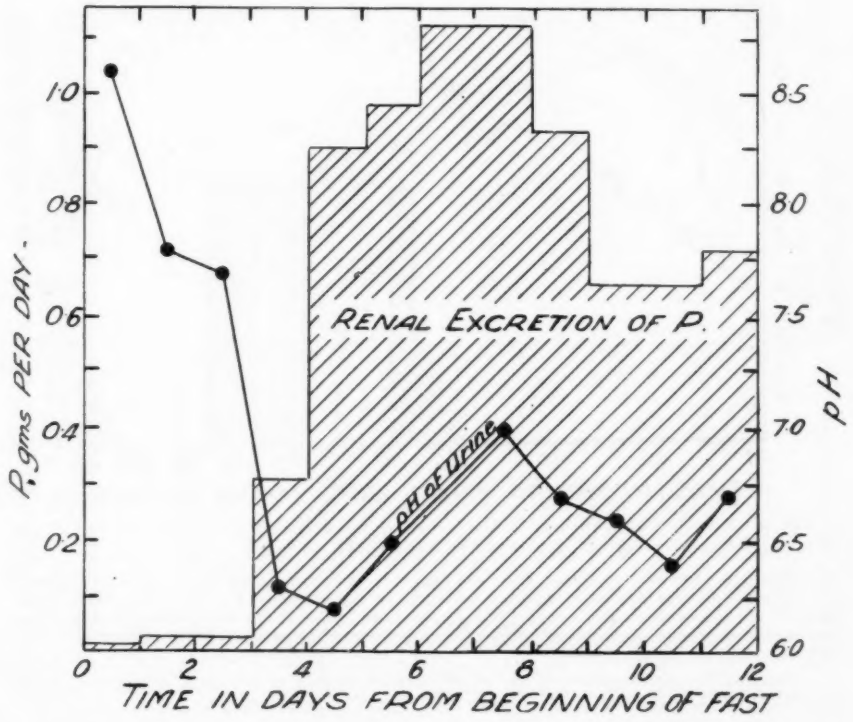

Fig. 3. The pH of the urine and the renal excretion of phosphorus during starvation. Sheep W_2–40.

An experiment was also made in which the pH of the urine and the rate of renal excretion of phosphorus in a fasting sheep were measured[3] (fig. 3). The pH was determined colorimetrically, and, unfortunately, no precautions could be taken to avoid loss of CO_2.

The pH of the urine fell from 8·6 to 6·3 in the first four days of starvation; coincidentally, the daily excretion of phosphorus rose from 0·01 to 0·3 gm.

Observations on the Renal Excretion of Phosphorus during Starvation carried out at more frequent intervals.

In the light of the apparent general relationships of the concentration of inorganic phosphate in the blood and the pH of the urine to the renal excretion of phosphorus during starvation, an attempt has been made to subject them to closer investigation. Blood and urine were collected simultaneously, at intervals, from a fasting sheep. The bladder was washed out with warm sterile 0·85%

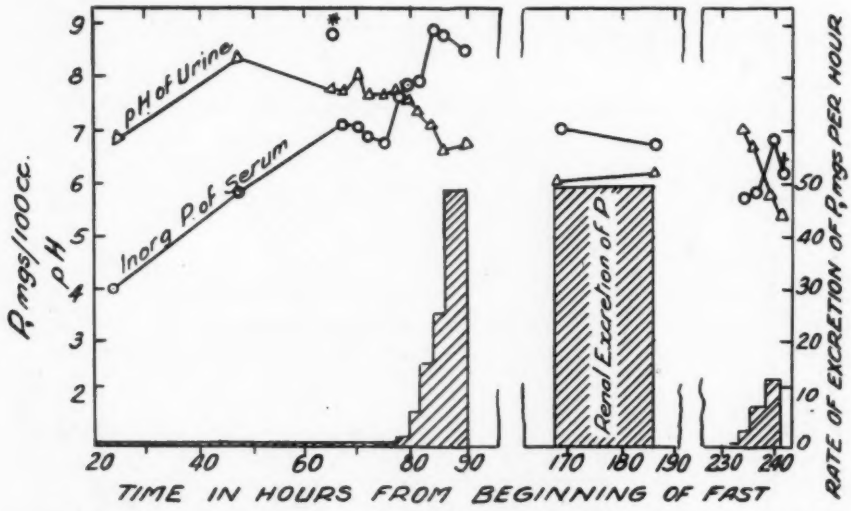

Fig. 4. Frequent observations on the inorganic phosphate of the serum, the pH of the urine, and the renal excretion of phosphorus during fast. Sheep W_1–36.

* This point has been excluded from the graph on the ground that in previous experience such a high concentration of inorganic phosphate in the serum had never been observed at this early stage in starvation; and, further, the change in concentration between this and the next observation was greater than was observed at any other time. No analytical error, however, could be detected.

† This fall in concentration is due to the administration of 200 gms. of cane sugar at 234–236 hours.

[3] My thanks are due to Mr. H. R. Marston for allowing me to put one of his starvation experiments to this use.

salt solution, and the washings added to the urine after the determination of pH, for which less than 0·5 c.c. of urine was used. Both blood and urine were collected under paraffin. The inorganic phosphate of the serum, and the quantity, pH, total phosphorus, and, where possible, inorganic phosphate of the urine were determined.

These observations were made over 90 hours, during which the concentration of inorganic phosphate in the serum increased from 4·0 to 8·9 mgs. P/100 c.c. After it reached about 7 mgs. P/100 c.c. they were made every two hours. They were suspended over the week-end, and resumed at 168 hours. The sheep was fed at 199 hours with lucerne chaff. At 216 hours, after it had eaten 200 gms., the food was again removed, and they were made during a second period of inanition.

The data of this experiment are set forth in fig. 4.

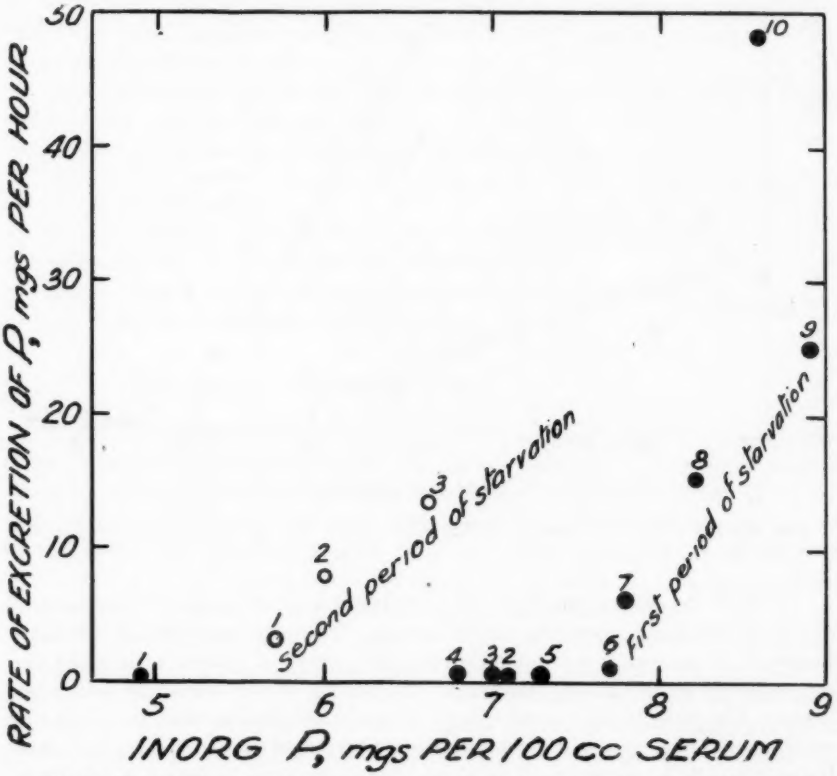

Fig. 5. The relation of the inorganic phosphate of the serum to the rate of excretion of phosphorus. Sheep W_1–36.

The figures indicate the sequence of the observations.

As the concentration of inorganic phosphorus in the urine is usually too low (if any is present) for reliable determination, a complete set of observations was obtained for total phosphorus only. Estimations of both total and inorganic phosphorus, where they could be reliably made, showed that, though organic phosphorus was always present, its concentration was less than 4 mgs. P/100 c.c. urine, so that there was but little difference between the excretion of total and inorganic phosphorus.

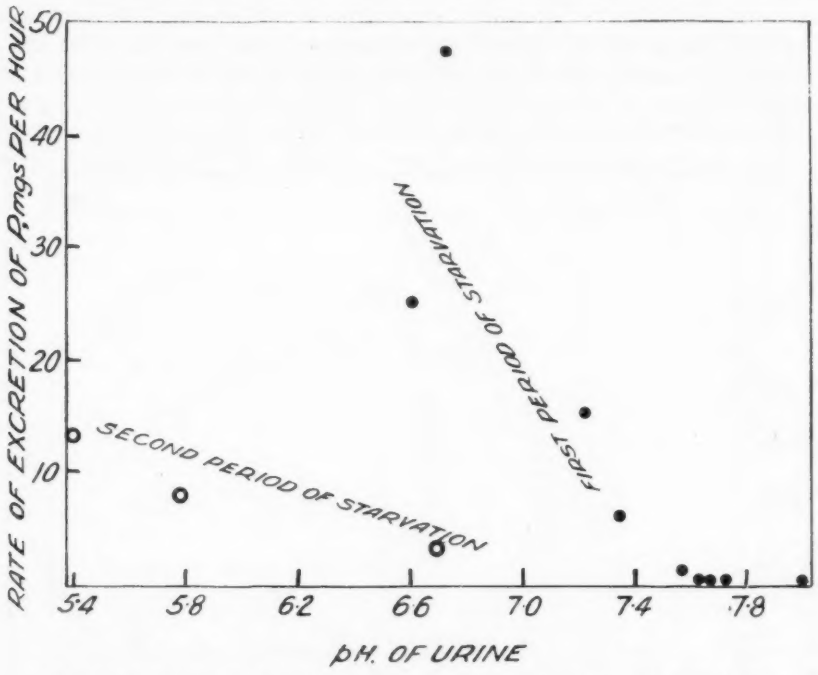

Fig. 6. The relation of the pH of the urine to the rate of excretion of phosphorus. Sheep W_1–36.

In fig. 5 the rate of excretion of phosphorus is plotted against the concentration of inorganic phosphate in the serum. The concentration of inorganic phosphate in the serum corresponding to each period of collection of urine has been taken from the graph (fig. 4) at the middle of the period of collection. Owing to the urine in the "dead space" of the uriniferous system, the period of collection of urine does not correspond with period of secretion of that urine. Himsworth (1931) considers the "dead space" to have a normal capacity of about 5 c.c. in the human, and if it be taken to be of the same order in the sheep,

the values for the inorganic phosphate of the serum at the middle of the computed periods of secretion differ but little from those at the middle of the period of collection.

In the first 77 hours of fast a trace of phosphorus was being excreted, the rate being independent of the concentration of inorganic phosphate in the serum, but after this for nine hours the rate of excretion was roughly proportional to the concentration of inorganic phosphate in the serum above 7·5 mgs. P/100 c.c. serum. The rate of excretion then still continued to rise, notwithstanding that the concentration of inorganic phosphate in the serum was falling, and in the observations made at 168–186 hours (fig. 4) considerable quantities of phosphorus were excreted at a concentration of inorganic phosphate in the serum below 7·5 mgs. P/100 c.c. At 234 hours the concentration of inorganic phosphate in the serum was 5·7 mgs. P/100 c.c., and only a trace of phosphorus was present in the urine. As the second period of inanition advanced, phosphorus was excreted in the urine at a rate roughly proportional to the concentration of inorganic phosphate in the serum above 5·3 mgs. P/100 c.c.

The relation between the rate of excretion of phosphorus and the pH of the urine during starvation is shown in fig. 6.

As the rate of excretion increases so the pH falls.

A further experiment was performed to decide whether these simultaneous changes in pH and rate of excretion of phosphorus were concomitant or causally related. Observations were made on a sheep which was fed ammonium chloride. It received 30 gms. per day in the form of a drench.

In two days the pH of the urine was reduced from 7·5 to 5·5. No inorganic phosphate, however, appeared in the urine.

DISCUSSION.

It has been pointed out in the introduction that usually only a trace of phosphorus is present in the urine of sheep. In the author's experience of merino sheep on Australian pastures, or pen-fed on chaff diets, the amount is of the order of 1–2 mgs. per cent., of which less than 0·5 mg. is inorganic. This small amount is excreted independently of the concentration over a wide range of concentrations, and probably consists for the most part of nucleo-proteins derived from the wear and tear of the epithelial lining of the urinary tract.

In the work recounted above, when the concentration of inorganic phosphate in the blood was raised somewhat above that found under ordinary conditions, phosphates were secreted in the urine. The rate of secretion in W_1–36 while fasting was at first roughly proportional to the concentration of inorganic phosphate in the serum in excess of 7·5 mgs. P/100 c.c., though later considerable quantities of phosphate continued to be secreted at values below this level, a sequence of events which has been found by others to occur also in the renal

excretion of glucose. During the second period of W_1–36's fast, the rate of secretion was roughly proportional to the concentration in the serum in excess of 5·3 mgs. P/100 c.c. In W_3–96 phosphate was not secreted in the urine until the concentration of inorganic phosphate in the *blood* was about 8 mgs. P/100 c.c. The data presented in fig. 1 and Table 1 indicate that a threshold, the level of which varied with the individual, existed in these sheep. Thus it would seem that there is a renal threshold for the excretion of inorganic phosphates in the sheep, but that it varies both in the one individual under different conditions and between individuals.

Though the secretion of phosphate during starvation was accompanied by a fall in the pH of the urine, the two happenings do not seem to be causally related, for when in another sheep the acidity of the urine was increased by the administration of ammonium chloride this did not bring about a secretion of phosphates. They can both be explained by increased tissue break-down.

The relatively high renal threshold for inorganic phosphate in sheep permits of a considerable range in the concentration of inorganic phosphate in the blood. On experimental diets containing variable amounts of easily assimilable phosphorus, DuToit, Malan and Rossouw (1930) showed that the concentration varied widely according to the amount obtainable in the diet, and this has been confirmed in this laboratory. Even on the one pasture, however, the author has obtained values ranging between 2·8 and 6·2 mgs. P/100 c.c. blood, the same sheep on different occasions sometimes approximating to each of these values.

SUMMARY AND CONCLUSIONS.

Only a trace of phosphorus has been found in the urine of sheep on pasture or chaff diets. The excretion of this trace of phosphorus was independent of the concentration of inorganic phosphate in the blood over a wide range of concentrations.

In sheep which had much higher concentrations of inorganic phosphate in the blood, consequent upon the addition of a gram of calcium phosphate to the daily ration, inorganic phosphate was secreted in the urine.

In fasting sheep the concentration of inorganic phosphate in the blood increased during the first few days. When the concentration reached about 7·5 mgs. P/100 c.c. serum, phosphates began to be secreted in the urine.

For some hours after it commenced the rate of secretion was in rough proportion to the concentration of inorganic phosphate in the serum above 7·5 mgs. P/100 c.c., but later the kidney secreted with greater efficiency.

The kidney still continued to secrete phosphates after the concentration of inorganic phosphate in the blood had fallen considerably below 7·5 mgs. P/100 c.c. serum.

The secretion of phosphate during starvation was associated with a fall in

the pH of the urine. Evidence is adduced to indicate that they are but concomitant phenomena.

It is concluded that in the sheep there is a renal threshold for inorganic phosphate above which it is secreted in the urine, but that the level of this threshold is variable, both in the one sheep under different conditions and in different sheep.

It has been pointed out that the high renal threshold permits of a considerable range of concentrations of inorganic phosphate in the blood.

ACKNOWLEDGMENTS.

My thanks are due to Mr. H. R. Marston for suggesting this investigation and for his valuable advice and assistance throughout the course of the work, and to Mr. C. E. Sleigh for his ready help with the sheep during the silent watches of the night. I am again indebted to Sir Charles Martin for his advice and very helpful criticism. Again, too, I wish to thank him and the Commonwealth Council for Scientific and Industrial Research for the hospitality extended me at the Division of Animal Nutrition.

REFERENCES.

Addis, T., Meyers, B. A. and Bayer, L. (1925): Amer. J. Physiol., 72, p. 125.
Brain, R. T., Kay, H. D. and Marshall, P. G. (1928): Biochem. J., 22, p. 628.
Cavins, A. W. (1924): J. Biol. Chem., 59, p. 237.
DuToit, P. J., Malan, A. I. and Rossouw, S. D. (1930): 16th Rep. Dir. Vet. Serv. and Anim. Ind., Dept. of Agric., U. of S. Africa, p. 313.
Fiske, C. H. and Subbarow, Y. (1925): J. Biol. Chem., 66, p. 375.
Forbes, E. B. and Keith, M. H. (1914): "Phosphorus Compounds in Animal Metabolism," Ohio Agric. Exper. Sta. Tech. Series, Bull. 5, p. 212.
Gouin, A. and Andouard, P. (1907): Soc. de l'aliment rationelle du bétial, Compt. rend. 11th congr., 11 and 127; cited by Forbes and Keith (1914).
Himsworth, H. P. (1931): Biochem. J., 25, p. 1128.
Maizels, M. and Hampson, A. C. (1927): J. Physiol., 63, Proceedings of the Physiol. Soc., March 19.
Walker, B. S. (1932): J. Lab. clin. Med., 17, p. 347.
Weiske, H. (1872): Z. Biol., 8, p. 246; cited by Forbes and Keith (1914).
Wilder, T. S. (1929): J. Biol. Chem., 81, p. 65.

THE BACTERIOLOGICAL OXIDATION OF SULPHUR IN AN AUSTRALIAN SOIL

by

PHYLLIS M. ROUNTREE[1]

(Waite Institute, University of Adelaide).[2]

(Submitted for publication 3rd July, 1933.)

The fact that the oxidation of elementary sulphur to sulphuric acid in soil is a bacteriological process has been well established for many years. Winogradsky (1887), Nathansohn (1902), Beijerinck (1904), and Trautwein (1921) studied the growth in culture media of various members of that group of autotrophic micro-organisms which is capable of obtaining the energy necessary for growth by the oxidation of sulphur and other partially oxidized sulphur compounds, such as thiosulphates. Brioux and Guerbet (1913) and Demolon (1913) showed that larger amounts of sulphur were oxidized in unsterilized soils than in sterile soils. Waksman and Joffe (1922) isolated from soil treated with flowers of sulphur an organism, *Thiobacillus thiooxidans*, capable of oxidizing sulphur with the production of large quantities of sulphuric acid. The acid produced by these organisms may serve as a solvent for insoluble minerals such as rock phosphate, as shown by McLean and Lint (1916), or may be used to neutralize an excess of bases in alkaline soils. Lipman (1916) and Hibbard (1921) suggest the application of elementary sulphur as a method for neutralizing alkaline soils. Rudolfs (1922) showed the effect of the application of sulphur to two alkaline soils of pH 9·2 and pH 9·6. These soils, treated with sulphur at the rate of 3,000 lbs. per acre, made up to a moisture content of 55% saturation and incubated at 28°C., reached neutral point after 12 weeks' incubation. The soils thus treated assumed a new set of physical properties characterized by more or less complete flocculation, and change in water-holding power and apparent specific gravity. Joffe and McLean (1924) investigated the effect of sulphur on the physico-chemical, chemical, and biological properties of an alkali soil. Their soil had an original pH value of 8·2, and this was reduced to neutrality after 95 days by an application of sulphur at the rate of 6,000 lbs. per acre. They concluded that the effect of the sulphur was to provide H ions, which replaced the Ca-Na cations of the exchangeable bases in the soil. The effect of the sulphur on the heterotrophic bacterial flora of the soil was to reduce the

[1] Research Student, Science and Industry Endowment Fund, Australian Council for Scientific and Industrial Research.

[2] Contribution from the Soils Division, Council for Scientific and Industrial Research.

bacterial numbers after 140 days' incubation. Previous to this date no change in numbers occurred, and they explained the decrease as being due to the increased concentration of salts in the soil due to sulphur oxidation.

The object of the investigations here reported was to determine the effect of elementary sulphur on an alkaline South Australian soil. The soil used was the Renmark clay loam,[3] which has a pH value of 8·4 and a high salt content. Samples were obtained from the first 9 inches of soil on Section 141, Block E, Renmark.

METHODS.

0·2% of precipitated sulphur was added to the soil,[4] which had been air dried, ground, and passed through a 2 mm. mesh sieve. The soil was distributed into jars in 100 gm. (calculated to oven dry weight) portions, and made up to the required moisture content by the addition of sterile distilled water. Sterilized soils were heated in the autoclave at 120°C. for one hour on two successive days, before the addition of the distilled water. The moisture-holding capacity of the soil was determined by Keen and Raczkowski's method (1921). All pH values were determined with the antimony oxide electrode.

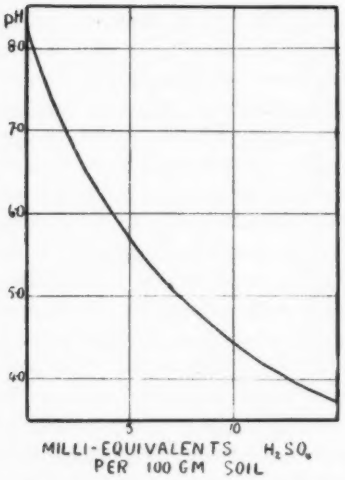

Fig. 1. The Buffer Curve of the Renmark clay loam, surface horizon.

The buffer capacity of the soil was determined by adding various amounts of N/10 H_2SO_4 to the air-dried soil, evaporating to dryness over a water bath, and determining the resultant pH values. From the buffer curve so obtained, which is shown in fig. 1, the amount of sulphur oxidized at a given pH value can be calculated, since the sulphur oxidized is converted directly to sulphuric acid. The reliability of the buffer curve was confirmed by the determination of the conductivities of a series of sulphur-treated soils. When the conductivities of these soils were plotted against the amounts of sulphur oxidized, as calculated from the buffer curve, the results fell along a straight line. All soils, except those in the experiment on the effect of varying temperature, were incubated at 25°C.

The principal medium (Medium No. 1) used for the cultivation of the bacteria isolated from sulphur-treated soils had the following composition: Sodium thiosulphate, 2·0 gms.; ammonium chloride, magnesium chloride, and mono-calcium phosphate, each, 0·1 gm.; agar, 25 gms.; distilled water, 1,000 mls. This medium has a pH of 6·0, and is a modification of that used by Waksman (1922). A liquid medium (Medium No. 2), containing precipitated sulphur, and similar to that used by Waksman (1922) for the cultivation of *Thiobacillus thiooxidans*, except that the pH was adjusted to 7·0 by using K_2HPO_4 instead of KH_2PO_4, was also employed. Bacterial counts were made by the dilution

[3] Formerly Type 6A, Taylor and England, 1929.

[4] Finely ground mineral sulphur was found to be oxidized as rapidly as precipitated sulphur.

method, five plates being poured from each final dilution. All plates and cultures were incubated at 25°C. The sulphur oxidizing power of all organisms isolated from the soil was determined by inoculating them into sterilized soils, the pH values of which were determined after 7 days' incubation.

FACTORS INFLUENCING SULPHUR OXIDATION IN SOIL.

Preliminary experiments showed that in a soil made up to a moisture content of 57% saturation, the rate of oxidation of sulphur, as measured by the change in pH values, was extremely rapid, the soil changing from pH 8·4 to pH 5·5 in 10 days. No change occurred in soil which had been sterilized, thus establishing that the change was due to a biological agency.

Influence of Soil Moisture.

An experiment was made to determine the influence of varying amounts of soil moisture on the rate of sulphur oxidation in the Renmark clay loam. The amount of water necessary for complete saturation of 100 gms. of the soil was found to be 43 mls., and a series of jars was set up containing 100 gms. of soil, 0·2% of sulphur, and amounts of water varying from 12 to 43 mls., that is, from 28% to 100% saturation. After incubation for 7 days the pH values of the soils were determined.

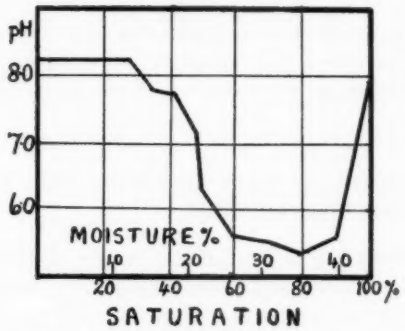

Fig. 2. Effect of soil moisture conditions on the rate of sulphur oxidation in unsterilized soil, incubated at 25°C. for 7 days.

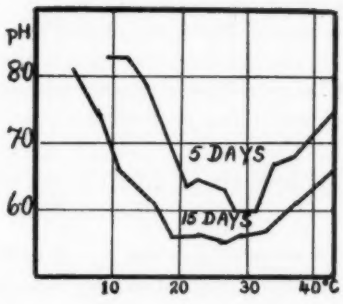

Fig. 3. Effect of varying temperature on sulphur oxidation in unsterilized soil at 57% saturation.

The results obtained are shown graphically in fig. 2. Sulphur is oxidized slightly at 35% saturation. but not at 28%. There is a wide optimum from 50% to 90% saturation, while no change occurs when the soil is completely saturated. McLean (1918) and Joffe (1922) state that from 50% to 60% saturation is the optimum moisture content for sulphur oxidation in sulphur, rock phosphate, and soil mixtures. It appears that in the Renmark clay loam, sulphur can be

rapidly oxidized over a much wider moisture range. In 7 days, at a moisture content of from 60% to 90% saturation, the pH will change from 8·4 to 5·6.

Results with sterilized soils inoculated with a mixed culture of bacteria isolated from treated soil show the same wide optimum. In such inoculated soils the rate of oxidation is even more rapid, the pH value of a soil moistened to 70% saturation changing from 8·4 to 4·6 in 7 days.

Influence of Soil Temperature.

In order to determine the effect on the rate of sulphur oxidation of varying soil temperature, a series of jars was set up, each containing 100 gms. of soil, 0·2 grm. of sulphur, and 24 mls. of water (57% saturation). These jars were incubated in a multiple temperature thermostat at temperatures ranging from 4°C. to 41°C. After incubation periods of 5 and 15 days, samples were withdrawn for pH determinations.

Fig. 3 sets out the results obtained. The graphs show that after 5 days' incubation the optimum falls between 21°C. and 33°C. There is no change in pH below 12°C., and above 34°C. the rate of oxidation slows down, although oxidation does not completely cease at 41°C., since in 5 days at that temperature the pH of the soil has fallen to 7·4. After 15 days' incubation the optimum range has widened out considerably, and falls between 16°C. and 35°C. At 8°C. some sulphur oxidation has occurred, and at 41°C. the pH has changed to 6·5. From these data it may be concluded that the organism responsible for sulphur oxidation in the Renmark clay loam is effective over a wide range of temperatures, and that the retarding effect of temperatures above and below the optimum is diminished over a long incubation period.

COURSE OF SULPHUR OXIDATION IN UNSTERILIZED AND IN STERILIZED INOCULATED SOIL.

A series of jars was set up to follow from day to day the course of sulphur oxidation in unsterilized soil and in sterilized soil inoculated with a sulphur oxidizing bacterium isolated from the Renmark clay loam. A series of sterilized soils was inoculated with 3-day cultures of this organism. The cultures were grown on thiosulphate agar (Medium No. 1), and the growth of each slant was emulsified with 25 mls. of sterile distilled water. This emulsion, when added to the soil, was sufficient to make the moisture content of 100 gms. of soil up to 65% saturation. The unsterilized soils were moistened up to 65% saturation in the usual way. Sterilized soils moistened with sterile distilled water served as controls. Immediately after inoculation thiosulphate agar plates were poured from a 1 in 1,000,000 dilution of an inoculated soil, in order to determine the number of organisms added per gm. of dry soil. The soils were incubated at 25°C., and on 8 successive days the pH values of the inoculated and of the un-

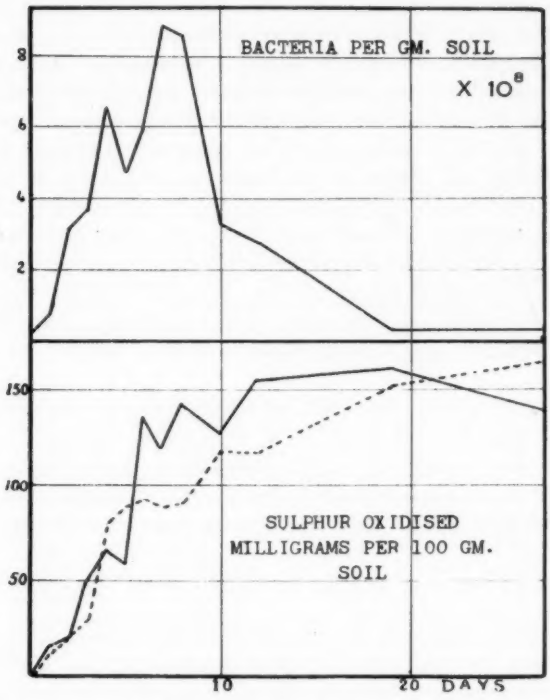

Fig. 4. Bacterial numbers and amount of sulphur oxidized in sterilized inoculated soil, and amount of sulphur oxidized in unsterilized soil at 65% saturation.

Broken line = unsterilized soil.
Continuous line = sterilized inoculated soil.

TABLE 1.

Sulphur Oxidation in Renmark Soil. 65% Saturation.

	Unsterilized Soil.			Sterilized Soil—Inoculated with Thiobacillus sp.			
Days.	pH values.	Milli-equivalents H_2SO_4 per 100 gms. soil.	Equivalent in mgs. S oxidized.	pH values.	Milli-equivalents H_2SO_4 per 100 gms. soil.	Equivalent in mgs. S oxidized.	Bacterial numbers Millions per gms. soil.
0	8·4			8·4			16
1	7·8	0·7	11·2	7·5	1·1	15·6	75
2	7·4	1·2	20·0	7·4	1·2	20·0	317
3	7·0	1·8	29·6	6·4	3·0	48·8	368
4	5·7	4·9	78·4	6·0	4·1	66·4	660
5	5·6	5·4	87·2	6·1	3·7	59·2	466
6	5·4	5·7	91·2	4·7	8·7	139·2	591
7	5·6	5·4	87·2	5·0	7·4	118·2	878
8	5·5	5·5	88·8	4·7	8·8	141·6	849
10	5·0	7·4	118·2	4·9	7·9	126·4	326
12	5·0	7·4	118·2	4·5	9·7	155·2	273
19	4·6	9·5	152·8	4·4	10·0	160·8	36
27	4·4	10·3	164·8	4·7	8·7	139·2	32

sterilized soils were determined, and bacterial counts made on the inoculated soils. After this period estimations were made after 10, 12, 19, and 27 days' incubation. The results obtained are shown in Table 1 and in fig. 4.

In the inoculated soil the bacterial numbers increased rapidly until the 7th day, rising from 15 millions per gm. of soil to 878 millions. The peak period on the 7th and 8th days corresponds to the point where the pH of the soil had reached 4·6. After this maximum the numbers fell away to 36 millions on the 19th day, and only a very small amount of sulphur was oxidized. From the 19th day onward practically no change occurred in pH value or bacterial numbers. The acidity of the soil at pH 4·6 seems to be the factor limiting bacterial activity, since there still remained in the soil 40 mgs. of unoxidized sulphur. Exhaustion of phosphorus and nitrogen supplies cannot be the limiting factor, since the same standstill is reached in liquid cultures containing an adequate supply of these elements.

In unsterilized soil the oxidation of sulphur follows the same trend as in inoculated soil, but the rate of oxidation appears to be slightly slower. The explanation of this slower rate of oxidation may be found in the fact that in unsterilized soil the sulphur oxidizing organism has to compete with other members of the bacterial flora for nutrient substances such as nitrogen and phosphorus.

ISOLATION OF SULPHUR OXIDIZING BACTERIA.

The organism used for the inoculation of sterilized soil in the above experiment was isolated from a sample of the Renmark clay loam, which had been treated with sulphur, and which had a pH value of 5·0. There is present in such a soil a number of bacteria which will grow on thiosulphate agar, but which do not oxidize sulphur when inoculated into sterile soil. The best method of eliminating these is by an enrichment process in sterilized soil. 0·1 gm. of treated soil was inoculated into a flask containing 100 mls. of Medium 2, and this was incubated at 25°C. for 6 days. 1 ml. of this culture in 20 mls. of sterile distilled water was used to inoculate a sterilized soil. After 6 days' incubation, 20 mls. of a 1 in 10 dilution of this soil was used to inoculate a second sterilized soil. This in turn was used to inoculate a third soil. Examination of thiosulphate agar plates poured from this third soil after 6 days' incubation showed that one type of colony was predominant. Cultures from such colonies oxidized sulphur when inoculated into sterilized soil. Enrichment of the sulphur oxidizing bacteria by passage through a series of sterilized soils has a depressive effect on all organisms other than those responsible for the oxidation of sulphur, and so facilitates the isolation of the latter in pure culture.

Four other organisms were isolated from thiosulphate agar plates, on which they grew abundantly, but when inoculated into sterilized soil they had no effect on sulphur. These organisms may possibly play a secondary rôle in sulphur

oxidation, by oxidizing any thiosulphates produced by the sulphur oxidizing bacteria. That the sulphur oxidizing bacterium is normally present in the Renmark clay loam was shown by adding 0·2% of sterilized sulphur to unsterilized soil, moistening to 65% saturation, and incubating. In 7 days approximately the same amount of sulphur was oxidized in this soil, as was oxidized in unsterilized soil mixed with unsterilized sulphur.

DESCRIPTION OF THE SULPHUR OXIDIZING ORGANISM ISOLATED FROM THE RENMARK CLAY LOAM.

Morphology. Three days' old culture on thiosulphate agar (pH 5·8); long, thin bacillus with rounded ends; 2–3μ in length, 0·4μ in breadth; no spores; no capsules. Non-motile in liquid media containing sulphur. No flagella could be demonstrated. Gram negative. Stains well with carbol erythrosine, carbol fuchsin, methylene blue, and methyl violet 6B. With methylene blue and carbol erythrosine the organisms have a distinctly beaded appearance, but are negative to Neisser's stain.

Cultural Characters. On thiosulphate agar plates, 4 days old, surface colonies round, smooth, shining; edges even, light to dark lemon-yellow in colour. Each colony contains a thick central nucleus which is dark yellow in colour, and which is surrounded by a lighter translucent zone. In older colonies (10 days) the dark central part constitutes the greater part of the colony, which appears dark yellow to the naked eye. The colonies vary from 0·25 to 0·5 mm. in diameter, with an average size of 0·4 mm. Colonies in the depth of the medium are chiefly lens-shaped, but may be round or oval, and present the characteristic appearance of the dark centre and lighter outer zone. The lens-shaped colonies average 0·4 mm. by 0·26 mm. in size, and in old colonies the light outer zone is 0·026 to 0·065 mm. in width. In plates poured from soil dilutions the colonies in the depth are only visible after 5 days' incubation. There is no separation of sulphur from the medium.

On thiosulphate agar slants growth is visible after 2 days' incubation; translucent, smooth, and shining; uniformly lemon-yellow. There is a tendency for the growth to coalesce with the medium.

On sulphur + mineral salt solution (pH 7·4). Growth good in 4–5 days, as shown by the turbidity of the medium; no pellicle; no sediment. The cultures take 3 weeks to change to a pH of 5·5. No growth on beef extract agar (pH 5·8). No growth on Thornton's asparagin agar (pH 7·0).

SYSTEMATIC RELATIONSHIPS OF THE RENMARK SULPHUR OXIDIZING ORGANISM.

Consideration of the strictly autotrophic nature of the organism isolated from the Renmark clay loam leads to the conclusion that it must be classed as a member of the genus *Thiobacillus*. It falls into that group of the Thiobacilli which acts primarily on elementary sulphur, oxidizing it rapidly to sulphuric acid, and appears to be closely related to *Thiobacillus thiooxidans*. Table 2 sets out the main points of difference and of similarity between the Renmark organism and *Thiobacillus thiooxidans* and *Thiobacillus trautweinii*.

TABLE 2.

	Thiobacillus thio-oxidans.	Thiobacillus trautweinii.	Thiobacillus sp.
Source of energy	Elementary sulphur thiosulphates	Thiosulphates	Elementary sulphur, thiosulphates
Size	$1 \times 0{\cdot}5\mu$	$1{\cdot}3\mu \times 0{\cdot}4\mu$	$2{-}3\mu \times 0{\cdot}4\mu$
Staining	Gram +ve	Gram —ve	Gram —ve
Accumulation of sulphur outside cell	None	None	None
Pellicle formation	None	None	None
Carbon sources	CO_2 from atmosphere	CO_2, organic compounds	CO_2 from atmosphere
Growth on organic media	None	Good	None
Motility	±	++	—
Acid accumulation	Very strong—pH goes down to 0·6	None—end products may be sulphate, dithionate, or tetrathionate	Strong—pH moves from 8·4 to 4·5 in soil, and to 5·5 in culture media
Limiting pH values	No growth above pH 6·0 Optimum pH 2·0–4·0	Limiting values pH 5·7, pH 11·5 Opt. 7·9–9·1	Grows well between pH 8·6 and 4·5 Optimum 5·0–6·0

Both *Thiobacillus thiooxidans* and the Renmark organism differ from *Th. trautweinii* in that the latter can grow heterotrophically on organic media. The Renmark organism differs from *Th. thiooxidans* in size, reaction to Gram's stain, and in the range of H ion concentration in which it is active. *Th. thiooxidans* will not grow above pH 6·0, and has its optimum between pH 2·0 and pH 4·0, that is, under very acid conditions. The Renmark organism will grow equally well under acid or alkaline conditions, but its action is limited by a pH of 4·5 in soil, since it cannot withstand any higher concentration of acid. These differences point to the fact that, although the Renmark organism is closely related to *Th. thiooxidans*, it should be regarded as a distinct species of the genus *Thiobacillus*. Cultures of a sulphur oxidizing bacterium identical with that isolated from the Renmark clay loam have been isolated from sulphur treated soils from Angaston, Cobdogla, Berri, and the Waite Institute, and so it may be concluded that this species of *Thiobacillus* has a wide distribution in the soils of South Australia.

DISCUSSION.

The chief factor contributing to the infertility of the Renmark clay loam is the high percentage of sodium contained in the exchangeable bases of this soil. It has been shown that elementary sulphur, when added to this soil under optimum conditions of temperature and moisture, is rapidly oxidized to sul-

phuric acid. The amount of sulphuric acid produced by the oxidation of 150 mgs. of sulphur in 100 gms. of soil is equivalent to one-third of the exchangeable bases present. The hydrogen ions of the H_2SO_4 replace the sodium in the soil colloid system, and the sodium can then be readily leached out from the soil as sodium sulphate. That the effect of the sulphur treatment is a lasting one is shown by the fact that pot soils treated with 0·1% of sulphur, and subsequently leached, had a pH value of 5·4 when sampled twelve months later. Those to which 0·2% of sulphur had been added had a pH value of 4·1 twelve months later. Soils so treated had their permeability to water greatly increased and were much easier to cultivate.

A striking feature of the oxidation of sulphur in the soil under investigation is the extreme rapidity with which the process is carried out. The amount of sulphur oxidized in 19 days under optimum conditions of temperature and moisture is equal to an application of 6,000 lb. per acre foot of soil. 0·03% of sulphur, or 1,200 lb. per acre foot, is sufficient to change the pH value of the soil from 8·4 to 7·0 in 3 days. The alkali soils treated by Joffe and McLean with a dressing of 6,000 lb. of sulphur per acre only reached neutrality after an incubation period of 13 weeks. Conditions affecting sulphur oxidation in Renmark clay loam appear to be much more favourable for rapid oxidation than are those existing in various American alkali soils that have been studied from this viewpoint.

SUMMARY.

The oxidation of elementary sulphur in an alkaline South Australian soil, Renmark clay loam, has been studied. The effects on the rate of sulphur oxidation of varying soil moisture content and of varying soil temperature have been determined. The optimum moisture content is found to lie between 60% and 90% saturation, and the optimum temperature between 16°C. and 35°C.

A member of the *Thiobacillus* group of soil bacteria has been isolated from sulphur treated soil, and its growth in sterilized soil studied. By its action the pH value of the soil is changed from 8·4 to 4·5 in 12 days, at which point the large amount of acid accumulated in the soil appears to be the factor limiting further growth. The chief morphological and cultural characters of the organism are described, and its systematic relationships discussed.

ACKNOWLEDGMENT.

The author wishes to express her thanks to Professor J. A. Prescott, of the Waite Agricultural Research Institute, for his valuable advice and criticism during the progress of the work.

REFERENCES.

Beijerinck, M. W. (1904): Centrbl. Bakt. II, 11, p. 593.
Brioux, Ch. and Guerbet, M. (1913): C.R. Acad. Sci., Paris, 156, p. 1476.
Demolon, M. A. (1913): C.R. Acad. Sci., Paris, 156, p. 725.
Hibbard, P. L. (1921): Soil Sci., 11, p. 385.
Joffe, J. S. (1922): Soil Sci., 14, p. 479.
Joffe, J. S. and McLean, H. C. (1924): Soil Sci., 17, p. 395.
Joffe, J. S. and McLean, H. C. (1924): Soil Sci., 18, pp. 13, 133, 237.
Keen, B. A. and Rackowski, H. (1921): J. Agr. Sci., 11, p. 441.
Lipman, J. G., McLean, H. C. and Lint, H. C. (1916): Soil Sci., 2, p. 499.
McLean, H. C. (1918): Soil Sci., 5, p. 251.
Nathansohn, A. (1902): Mitt. Zool. Station, Neapel, 15, p. 655.
Rudolfs, W. (1922): Soil Sci., 13, p. 215.
Taylor, J. K. and England, H. N. (1929): Austr. C.S.I.R. Bulletin No. 42.
Trautwein, K. (1921): Centrbl. Bakt. II, 53, p. 513.
Waksman, S. A. (1922): J. Bact., Baltimore, 7, p. 609.
Waksman, S. A. (1922): Soil Sci., 13, p. 329.
Waksman and Joffe, J. S. (1922): J. Bact., Baltimore, 7, p. 239.
Winogradsky, S. (1887): Bot. Ztg., 45, p. 489.

EXPERIMENTS ON THE GROWTH AND LONGEVITY OF THE WHITE MOUSE

2. THE INFLUENCE OF THE CONTINUOUS ADMINISTRATION OF VEGETABLE NUCLEIC ACID AND THE INTERMITTENT ADMINISTRATION OF THYROID UPON GROWTH AND LONGEVITY.

by

T. BRAILSFORD ROBERTSON, MARY C. DAWBARN,[1] J. W. WALTERS, AND J. D. O. WILSON

(From the Laboratory of the Division of Animal Nutrition of the Commonwealth Council for Scientific and Industrial Research at the University of Adelaide, South Australia).

(Submitted for publication 22nd February, 1933.)

Robertson (1928) published two papers on the influence on growth and longevity of nucleic acids of various origin and of thyroid administered separately or simultaneously to mice. This further series of experiments was planned by Professor Robertson, and has been completed by his collaborators.

The methods of selecting and caring for the animals and the principles underlying the construction of the graphs have been fully described in a preceding paper in this Journal (Robertson *et al.*, 1933).

Two complete experimental units were employed, each consisting of 36 male and 36 female animals receiving dietary supplements, and 36 male and 36 female animals, litter mates of the others, which served as normal controls.

The control animals were fed on the standard mixed diet described in the preceding paper. All the experimental mice received the same diet as the controls, plus 25 mgs. daily,[2] throughout life, of vegetable nucleic acid obtained from The British Drug Houses Ltd. They were also given a thyroid powder prepared by pulverizing Burroughs Wellcome 5 gr. thyroid tablets. The powder contained 0·095% iodine. The "experimental" male mice in Experiment A received 1·5 mg. of this thyroid powder ($\equiv$ 1·8 to 1·9 mg. of fresh thyroid) daily from 6 to 10 weeks of age. The females in the same experiment were given 3·0 mgs. of thyroid powder daily from 6 to 10 weeks of age. In Experiment B the male mice

[1] Research Chemist to The Animal Products Research Foundation of the University of Adelaide.

[2] "Daily" is used to mean "on six days a week."

received 1·5 mg. of thyroid powder daily from 6 to 10 weeks, and 3·0 mgs. daily from 26 to 30 weeks of age. The female animals were given 1·5 mg. of thyroid daily over three 28-day periods, from 6 to 10, 26 to 30, and 50 to 54 weeks of age.

As previously stated, the dietary supplements were always mixed with the milk and egg fraction of the diet, and were shared between the mice in a cage. In every case the feeding was so conducted that every animal received the thyroid at exactly the ages stated above.

The results of the experiments are shown graphically in figs. 1, 2, 3, and 4, and in tabular form in Tables 1 to 10.

ANALYSIS OF THE RESULTS.

Experiment A: Male Mice. The growth curve of the experimental animals was below that of the controls from the 27th to the 60th week of age; from the 60th to the 90th week the curves coincided, and thereafter the experimental curve was above the control.

The maximum divergence between the curves was 2·4 gms. at 50 weeks, when the mean weight of the control mice had reached its maximum. The mean maximum weight of the treated animals was at 68 weeks. The difference between the two maxima was 1·0 gm.

The survival curve of the experimental animals was at all points above that of the controls, and the mean duration of life was 59 days longer (probable error 26 days). The experimental animals also exhibited a delayed and diminished senescent loss of weight.

Experiment A: Female Mice. From the 14th to the 116th week of age the mean weight of the experimental animals was considerably less than that of the controls. The maximum difference, 4·0 gms., was at 64 weeks, when the controls reached, and the experimental animals approached, their maximum weight.

The survival curve of the treated animals was above that of the controls till 950 days (136 weeks) of age, and their mean duration of life was 54 days longer (probable error 25 days). Senescent loss of weight was diminished so that the widely diverging weight curves coincided from the 118th week.

Experiment B: Male Mice. The "experimental" growth curve was below the control for most of its length, though the distance between them was not great. From the 94th to the 134th weeks they overlapped, showing that there was no significant difference between the weights of the animals.

A temporary definite fall in weight occurred during the second period of thyroid administration.

The mean life duration of the treated animals was insignificantly greater than that of the controls.

Experiment B: Female Mice. The growth curve of the experimental animals was below that of the controls for the whole life-span, except from the 86th to

the 100th week, and from the 126th to the 134th weeks of age, when they were coincident.

The survival curve of the control was superior to that of the experimental mice, and the mean duration of life was longer by 51 days (probable error 27 days).

DISCUSSION OF THE RESULTS.

(1) *Influence of Vegetable Nucleic Acid and Thyroid on Growth.*

In his earlier experiments Robertson (1928) found that the daily administration of 25 mgs. of vegetable nucleic acid to male mice throughout their lives caused a slight retardation of growth from the 10th to the 30th week. Thereafter the growth curves of the control and experimental animals were virtually coincident till the 70th week, when the latter showed a tendency to rise above the other for about 20 weeks.

The growth of male mice in receipt of 1·5 mg. daily throughout life, of the same thyroid powder as that used in the present experiments, was superior to that of the control animals from the 10th to the 70th week, though the maximum weight attained was not significantly greater.

Cameron and Carmichael (1920) fed thyroid to young rats for periods of from 17 to 76 days, and in every case noticed a retardation in growth rate. The smallest amount fed each day was in 1 in 20,000 of the body weight of the animals of a thyroid preparation containing 0·39% iodine. The amount fed by Robertson was ·0001 of the body weight of the animals when they weighed 15 gms., ·00005 when they weighed 30 gms., of a preparation containing 0·095% iodine.

The matter was discussed by Robertson (1928), and reasons for the difference between the results obtained by him and by Cameron were suggested.

The fact that Cameron's thyroid powder contained four times as much iodine as that used in these laboratories must have been a contributory cause.

Cameron and Carmichael (1922, 1924) found that while in receipt of a thyroid preparation containing 0·34% or 0·38% iodine (1 part : 5,000 of body weight) young rats gained weight more slowly than their controls, but that after the thyroid feeding ceased they showed a definite, though only temporary, acceleration of growth.

In the present experiments the feeding of thyroid from the 6th to the 10th week of age, to animals in receipt of vegetable nucleic acid, depressed growth from about the 15th week, the mean maximum weight of the treated animals being less than that of the controls in every group. A second or third period of thyroid feeding had no further effect on growth, except a temporary depression during the time of administration. The maximum effect was produced by feeding 3 mgs. daily for a single period from 6 to 10 weeks of age.

Thus neither vegetable nucleic acid nor thyroid, when fed separately and continuously to mice, diminished the weight attained by the animals.

But when vegetable nucleic acid was fed continuously, and thyroid for one, two, or three 28-day periods, the mean maximum weight of the animals was reduced below that of the controls.

(2) *Influence of Vegetable Nucleic Acid and Thyroid on Longevity.*

Vegetable nucleic acid was previously found to increase the mean life duration of male mice from 609 to 706 days.

The daily administration of 1·5 mg. of thyroid powder throughout life diminished the mean length of life from 728 to 623 days.

When male mice 550 days old were given a daily dose of 15 mgs. of spleen nucleic acid for the rest of their lives, and a daily dose of 3·0 mgs. of thyroid powder for six weeks, they at first died in rapid succession. By 700 days of age, however, the survival curves of treated and control animals coincided, and the expectation of life of the treated animals at this stage was 172 days, as compared with 77 days for the controls.

In Experiment A of the present series the life duration of the "experimental" male mice was increased by 59, and of the female mice by 54 days.

The increased longevity due to the treatment was less than with vegetable nucleic acid alone. That small intermittent doses of thyroid fed to *young* animals in receipt of vegetable nucleic acid have an injurious effect on longevity is confirmed by the fact that when administered over two 28-day periods the life duration of the treated animals was not significantly greater than that of the controls, and the mice that received thyroid over three 28-day periods had a shorter life duration than the controls.

SUMMARY.

The growth of mice in receipt of 25 mgs. daily of vegetable nucleic acid throughout life, and of small amounts of thyroid for one, two, or three 28-day periods, was reduced below that of the controls.

The depression of growth was most marked when 3·0 mgs. daily of a thyroid preparation containing 0·1% of iodine was fed to animals from the 6th to the 10th week of age. Lesser effects were produced

(1) when 1·5 mg. was fed from the 6th to the 10th week,
(2) when 1·5 mg. was fed from the 6th to the 10th week, and 3·0 mgs. from the 26th to the 30th week,
(3) when 1·5 mg. was fed from the 6th to the 10th, from the 26th to the 30th, and from the 50th to the 54th weeks.

When thyroid was fed for only one period, from the 6th to the 10th week, senescent loss of weight was diminished and mean life duration was increased.

This effect was negatived by a second period of thyroid administration and reversed by a third, so that life duration was diminished.

ACKNOWLEDGMENTS.

We would like to thank Sir Charles Martin for his most helpful criticism of these papers.

REFERENCES.

Cameron, A. T. and Carmichael, J. (1920): J. Biol. Chem., 45, p. 69.
Cameron, A. T. and Carmichael, J. (1922): Trans. Roy. Soc. Canada, 3rd series, 16, p. 57.
Cameron, A. T. and Carmichael, J. (1924): Trans. Roy. Soc. Canada, 3rd series, 18, p. 105.
Robertson, T. Brailsford (1928): Austral. J. exp. Biol., 5, pp. 47 and 69.
Robertson, T. B., Dawbarn, M. C., Walters, J. W. and Wilson, J. D. O. (1933): Austral. J. exp. Biol., 11, p. 99.

Experiment A. Table 1.

Growth of Control Male White Mice, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
36	5	9·26	·13	33	60	31·62	·51
36	6	10·64	·18	32	62	31·69	·50
36	7	12·28	·23	32	64	31·78	·54
36	8	13·50	·25	31	66	31·95	·49
36	9	15·25	·24	31	68	32·35	·51
36	10	16·56	·27	31	70	31·81	·54
36	11	18·06	·22	31	72	31·82	·53
36	12	19·01	·20	28	74	31·59	·57
36	13	19·90	·24	28	76	31·29	·59
35	14	21·01	·24	28	78	31·29	·55
35	15	21·49	·22	28	80	30·88	·54
35	16	22·27	·19	26	82	31·42	·53
35	17	22·81	·21	26	84	31·69	·55
35	18	23·66	·21	26	86	31·08	·45
34	19	24·21	·23	26	88	30·83	·44
34	20	24·65	·25	26	90	30·56	·46
34	21	24·88	·27	25	92	29·82	·47
34	22	25·49	·26	25	94	30·10	·44
33	23	25·67	·30	25	96	29·96	·44
33	24	26·12	·31	24	98	30·19	·40
33	25	26·14	·30	24	100	29·42	·46
33	26	26·11	·27	22	102	28·55	·43
33	27	26·48	·31	22	104	28·75	·42
33	28	27·24	·27	20	106	29·07	·45
33	29	27·38	·29	19	108	28·97	·47
33	30	27·42	·30	19	110	28·24	·46
33	32	27·98	·32	18	112	26·72	·56
33	34	28·92	·33	17	114	27·06	·53
33	36	29·23	·29	17	116	26·35	·54
33	38	30·03	·46	14	118	26·07	·46
33	40	30·53	·50	13	120	25·69	·52
33	42	31·05	·53	12	122	25·58	·45
33	44	31·27	·57	11	124	26·05	·50
33	46	31·86	·57	11	126	25·41	·43
33	48	32·36	·55	10	128	25·05	·48
33	50	32·92	·53	9	130	23·61	·69
33	52	32·89	·57	6	132	23·58	·79
33	54	32·65	·55	5	134	23·50	1·10
33	56	32·76	·54	4	136	24·37	—
33	58	32·24	·52	3	138	22·67	—

EXPERIMENT A. TABLE 1 (continued).

Growth of Control Male White Mice, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
2	140	23·70	—	1	148	23·00	—
1	142	24·00	—	1	150	22·50	—
1	144	24·50	—	1	152	23·00	—
1	146	23·00	—	1	154	22·00	—

EXPERIMENT A. TABLE 2.

Growth of Male White Mice Receiving Vegetable Nucleic Acid and Thyroid, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
35	5	9·56	·16	34	68	31·90	·44
35	6	10·47	·23	33	70	31·71	·47
35	7	11·41	·24	33	72	31·30	·52
35	8	12·64	·29	33	74	31·18	·50
35	9	14·44	·34	32	76	31·44	·49
35	10	16·39	·32	32	78	31·47	·52
35	11	18·67	·27	32	80	31·48	·52
35	12	19·66	·25	32	82	31·67	·49
35	13	20·81	·26	32	84	31·06	·52
35	14	21·53	·24	32	86	31·20	·50
35	15	22·03	·23	31	88	31·52	·48
35	16	22·39	·23	30	90	31·17	·50
35	17	22·53	·24	30	92	31·35	·47
35	18	23·26	·24	29	94	31·10	·53
35	19	23·77	·25	28	96	31·21	·45
35	20	24·23	·24	27	98	30·89	·49
35	21	24·40	·22	25	100	31·08	·47
35	22	24·77	·25	25	102	30·48	·45
35	23	25·43	·24	25	104	30·16	·50
34	24	25·63	·26	25	106	29·74	·53
34	25	25·68	·26	24	108	30·17	·41
34	26	25·90	·25	22	110	29·61	·54
34	27	26·24	·27	22	112	28·98	·50
34	28	26·46	·26	22	114	28·77	·56
34	29	26·28	·29	21	116	28·19	·50
34	30	26·56	·31	21	118	28·29	·52
34	32	27·40	·31	21	120	27·62	·55
34	34	27·84	·34	19	122	27·58	·48
34	36	28·29	·38	15	124	26·50	·55
34	38	28·81	·41	13	126	26·62	·59
34	40	29·35	·42	13	128	26·81	·61
34	42	29·31	·44	10	130	27·40	·49
34	44	30·04	·43	10	132	27·00	·52
34	46	30·18	·46	10	134	26·10	·69
34	48	30·29	·47	8	136	25·50	·77
34	50	30·54	·44	6	138	25·20	·84
34	52	30·54	·46	5	140	25·70	1·27
34	54	30·81	·47	4	142	26·50	—
34	56	31·16	·46	3	144	27·83	—
34	58	30·72	·40	3	146	26·33	—
34	60	30·99	·44	2	148	30·00	—
34	62	31·44	·46	2	150	26·25	—
34	64	31·12	·48	1	152	24·50	—
34	66	31·74	·46				

EXPERIMENT A. TABLE 3.

Growth of Control Female White Mice, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
36	5	8·74	·15	34	66	31·82	·66
36	6	10·42	·22	34	68	31·83	·66
36	7	11·56	·19	33	70	31·53	·63
36	8	13·26	·19	32	72	31·86	·64
36	9	14·57	·23	31	74	31·65	·67
36	10	15·86	·25	31	76	31·35	·68
36	11	17·13	·24	31	78	30·92	·69
36	12	17·97	·25	30	80	30·88	·68
36	13	18·76	·21	30	82	30·68	·63
36	14	19·74	·22	30	84	30·32	·63
36	15	20·26	·23	30	86	30·20	·62
36	16	20·69	·23	29	88	30·03	·61
36	17	21·14	·22	27	90	30·52	·59
36	18	21·79	·24	27	92	30·13	·59
36	19	22·07	·25	25	94	29·42	·69
36	20	22·51	·26	25	96	29·16	·66
36	21	22·88	·28	23	98	29·07	·68
36	22	23·22	·28	22	100	28·41	·80
36	23	23·33	·31	20	102	29·18	·90
36	24	23·78	·33	20	104	29·53	·89
36	25	23·99	·32	19	106	28·63	·84
36	26	24·29	·34	18	108	28·47	·92
36	27	24·82	·35	18	110	28·42	·88
36	28	25·08	·36	18	112	28·25	·84
36	29	25·28	·39	18	114	28·25	·80
36	30	25·46	·39	16	116	27·69	·88
36	32	26·31	·41	14	118	27·57	·87
36	34	27·11	·44	14	120	26·71	·92
36	36	27·67	·47	10	122	27·50	·81
36	38	28·31	·52	10	124	26·75	·75
36	40	29·18	·58	9	126	25·44	·79
36	42	29·93	·63	9	128	24·94	·73
36	44	30·68	·64	7	130	25·93	·59
36	46	30·60	·67	7	132	25·93	·48
36	48	31·04	·71	6	134	24·92	·48
36	50	31·35	·73	6	136	24·30	·69
36	52	31·26	·73	5	138	24·20	·92
36	54	31·10	·74	4	140	23·25	—
36	56	31·71	·73	2	142	26·25	—
35	58	31·93	·78	2	144	26·50	—
34	60	32·25	·79	2	146	25·50	—
34	62	32·22	·79	1	148	26·00	—
34	64	32·32	·74	1	150	23·00	—

EXPERIMENT A. TABLE 4.

Growth of Female White Mice Receiving Vegetable Nucleic Acid and Thyroid, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
34	5	9·29	·15	34	9	14·14	·29
34	6	10·54	·14	34	10	15·75	·37
34	7	11·44	·20	34	11	17·44	·28
34	8	12·37	·30	34	12	18·37	·26

EXPERIMENT A. TABLE 4 (continued).

Growth of Female White Mice Receiving Vegetable Nucleic Acid and Thyroid, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
34	13	18·81	·28	34	72	28·47	·43
34	14	19·07	·26	34	74	28·29	·45
34	15	19·43	·25	34	76	28·07	·47
34	16	19·96	·26	33	78	27·68	·47
34	17	20·40	·28	33	80	27·62	·46
34	18	20·57	·27	33	82	27·56	·46
34	19	21·35	·24	33	84	27·41	·52
34	20	21·63	·21	33	86	27·08	·52
34	21	21·91	·21	33	88	27·76	·54
34	22	22·25	·23	33	90	26·85	·58
34	23	22·57	·22	31	92	26·63	·52
34	24	22·68	·24	29	94	26·74	·56
34	25	22·90	·23	29	96	26·71	·63
34	26	23·19	·26	28	98	27·04	·59
34	27	23·49	·27	26	100	26·85	·53
34	28	23·75	·27	24	102	27·35	·55
34	29	23·81	·29	24	104	26·63	·57
34	30	24·19	·27	23	106	26·43	·56
34	32	24·60	·27	22	108	25·73	·67
34	34	25·32	·30	20	110	26·82	·66
34	36	25·74	·33	18	112	27·25	·69
34	38	25·93	·33	18	114	26·86	·68
34	40	26·31	·34	18	116	25·75	·69
34	42	27·31	·33	15	118	27·27	·59
34	42	27·31	·33	15	120	26·80	·50
34	44	27·60	·38	13	122	26·46	·68
34	46	27·81	·40	13	124	25·96	·68
34	48	28·10	·39	12	126	26·00	·66
34	50	28·29	·41	12	128	25·04	·68
34	52	28·06	·41	10	130	25·15	·70
34	54	28·13	·42	9	132	24·50	·74
34	56	28·31	·42	6	134	25·58	·85
34	58	28·43	·44	4	136	25·75	—
34	60	28·54	·44	4	138	26·00	—
34	62	28·54	·46	4	140	25·62	—
34	64	28·28	·44	3	142	27·33	—
34	66	28·68	·42	1	144	32·00	—
34	68	28·60	·42	1	146	29·50	—
34	70	28·16	·44				

EXPERIMENT B. TABLE 5.

Growth of Control Male White Mice, 1929-1933.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
36	5	9·03	·15	36	13	20·10	·24
36	6	10·35	·18	36	14	20·85	·24
36	7	12·28	·27	36	15	21·43	·24
36	8	13·96	·30	36	16	22·03	·26
36	9	15·60	·30	36	17	22·67	·26
36	10	16·75	·28	36	18	22·99	·25
36	11	18·11	·28	36	19	23·54	·25
36	12	19·32	·23	36	20	23·83	·25

EXPERIMENT B. TABLE 5 (continued).

Growth of Control Male White Mice, 1929-1933.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
36	21	24·15	·26	25	92	30·76	·50
36	22	24·29	·25	24	94	30·48	·51
36	23	24·86	·27	24	96	30·27	·53
36	24	25·32	·29	24	98	30·12	·53
36	25	25·61	·29	24	100	29·94	·48
36	26	25·78	·30	24	102	29·87	·50
36	27	25·97	·31	24	104	29·19	·49
36	28	26·19	·31	24	106	28·73	·52
36	29	26·46	·33	22	108	28·25	·53
36	30	26·64	·32	20	110	28·47	·41
36	32	27·68	·30	19	112	28·55	·48
35	34	28·21	·34	19	114	27·76	·54
35	36	28·21	·33	18	116	28·31	·41
35	38	28·59	·35	18	118	27·47	·43
35	40	28·89	·33	17	120	27·12	·53
35	42	29·30	·37	16	122	27·31	·40
35	44	29·84	·38	12	124	26·46	·47
35	46	30·34	·39	10	126	26·95	·74
35	48	30·61	·38	10	128	28·10	·64
35	50	30·27	·41	8	130	27·37	·89
35	52	30·01	·39	7	132	26·71	·95
35	54	30·11	·41	6	134	26·66	1·08
35	56	30·31	·36	5	136	27·40	1·21
35	58	30·10	·38	5	138	28·32	1·15
35	60	30·11	·37	5	140	27·60	·92
35	62	30·34	·38	5	142	26·80	·53
34	64	30·40	·39	5	144	27·30	·58
34	66	31·19	·40	5	146	27·60	·92
34	68	30·12	·42	5	148	24·90	1·11
33	70	31·17	·40	3	150	25·00	—
33	72	31·71	·45	3	152	24·33	—
32	74	31·45	·48	1	154	29·00	—
32	76	31·16	·42	1	156	28·50	—
30	78	30·28	·47	1	158	29·00	—
30	80	30·55	·48	1	160	26·50	—
29	82	30·21	·52	1	162	24·50	—
26	84	30·77	·46	1	164	25·50	—
26	86	30·31	·55	1	166	24·0	—
25	88	31·02	·56	1	168	21·5	—
25	90	30·96	·49				

EXPERIMENT B. TABLE 6.

Growth of Male White Mice Receiving Vegetable Nucleic Acid and Thyroid, 1929-1933.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
35	5	9·16	·14	35	12	18·80	·27
35	6	10·20	·21	35	13	19·71	·24
35	7	11·71	·28	35	14	20·30	·24
35	8	12·99	·26	35	15	20·83	·23
35	9	14·58	·29	35	16	21·46	·23
35	10	15·70	·31	35	17	22·17	·23
35	11	17·64	·30	35	18	22·67	·25

EXPERIMENT B. TABLE 6 (continued).

Growth of Male White Mice Receiving Vegetable Nucleic Acid and Thyroid, 1929-1933.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
35	19	23·09	·21	31	80	30·34	·33
35	20	23·44	·24	31	82	30·23	·36
35	21	23·99	·25	31	84	29·76	·36
35	22	24·24	·27	30	86	30·20	·36
35	23	24·39	·25	30	88	30·23	·33
35	24	24·56	·24	29	90	29·90	·35
35	25	25·10	·24	29	92	29·46	·38
35	26	25·26	·25	29	94	29·41	·47
35	27	24·90	·26	25	96	29·88	·47
35	28	24·79	·22	25	98	29·32	·47
35	29	25·00	·23	24	100	29·21	·56
35	30	25·63	·26	23	102	29·28	·43
35	32	27·10	·32	23	104	29·50	·43
35	34	27·41	·33	22	106	29·25	·38
35	36	27·64	·30	21	108	28·17	·47
35	38	28·03	·28	20	110	27·77	·49
35	40	28·36	·30	18	112	27·72	·61
35	42	28·70	·31	18	114	27·14	·74
35	44	28·69	·31	17	116	27·53	·64
35	46	29·66	·28	17	118	27·65	·59
35	48	29·54	·25	16	120	27·00	·53
35	50	29·21	·30	16	122	26·69	·62
35	52	28·89	·33	15	124	27·03	·52
34	54	29·34	·26	15	126	26·87	·58
34	56	29·43	·26	14	128	26·82	·38
34	58	29·16	·24	14	130	25·92	·46
34	60	29·59	·27	9	132	25·61	·56
34	62	29·81	·32	7	134	24·57	·79
34	64	29·75	·29	6	136	24·91	·46
34	66	30·03	·28	6	138	24·50	·58
34	68	30·26	·27	3	140	24·50	—
33	70	30·24	·34	3	142	23·16	—
33	72	30·65	·32	3	144	23·83	—
32	74	30·58	·40	3	146	24·67	—
32	76	30·39	·38	2	148	22·75	—
31	78	30·18	·29	1	150	22·50	—

EXPERIMENT B. TABLE 7.

Growth of Control Female White Mice, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
36	5	8·68	·14	36	15	19·24	·24
36	6	9·76	·17	36	16	19·79	·25
36	7	11·60	·21	36	17	20·04	·23
36	8	13·42	·20	36	18	20·50	·25
36	9	14·44	·23	36	19	21·03	·27
36	10	15·40	·25	36	20	21·38	·29
36	11	16·11	·22	36	21	21·61	·27
36	12	17·33	·21	36	22	22·01	·28
36	13	17·61	·22	36	23	22·08	·28
36	14	18·03	·25	36	24	22·43	·30

EXPERIMENT B. TABLE 7 (continued).

Growth of Control Female White Mice, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
36	25	22·97	·32	32	88	28·25	·60
36	26	22·94	·30	30	90	27·87	·61
36	27	23·32	·33	30	92	27·75	·63
36	28	23·69	·31	30	94	27·37	·69
36	29	23·72	·32	28	96	27·86	·63
36	30	23·88	·32	28	98	27·46	·65
36	32	24·57	·33	28	100	27·46	·66
36	34	24·79	·33	26	102	27·58	·69
36	36	25·22	·33	24	104	27·54	·69
36	38	25·35	·33	22	106	27·48	·70
36	40	25·75	·33	22	108	27·11	·77
36	42	25·94	·34	21	110	27·33	·78
36	44	26·44	·32	21	112	27·48	·72
36	46	26·79	·36	20	114	27·45	·71
36	48	26·74	·33	19	116	27·37	·61
36	50	26·85	·35	18	118	27·50	·47
36	52	26·96	·34	18	120	26·86	·44
36	54	27·21	·35	17	122	26·59	·61
35	56	27·50	·41	15	124	26·33	·59
35	58	27·73	·39	14	126	26·11	·52
35	60	27·70	·44	12	128	25·16	·53
33	62	28·11	·45	12	130	24·79	·59
33	64	28·09	·48	11	132	24·81	·63
33	66	27·70	·49	8	134	24·25	·70
33	68	28·45	·49	8	136	23·90	·92
33	70	28·47	·52	5	138	24·80	·71
33	72	28·70	·54	3	140	25·67	—
33	74	28·94	·51	2	142	24·75	—
33	76	29·29	·55	1	144	27·00	—
33	78	28·48	·55	1	146	26·00	—
33	80	28·79	·54	1	148	26·50	—
33	82	28·68	·62	1	150	27·00	—
33	84	28·55	·58	1	152	26·00	—
32	86	28·30	·61				

EXPERIMENT B. TABLE 8.

Growth of Female White Mice Receiving Vegetable Nucleic Acid and Thyroid, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
33	5	9·11	·17	33	18	19·48	·21
33	6	9·67	·22	33	19	20·15	·19
33	7	10·48	·25	33	20	20·52	·19
33	8	12·09	·26	33	21	20·65	·20
33	9	12·74	·27	33	22	20·86	·18
33	10	13·80	·31	33	23	21·26	·17
33	11	15·58	·21	33	24	21·68	·19
33	12	16·59	·21	33	25	22·08	·21
33	13	17·02	·20	33	26	22·08	·20
33	14	17·55	·20	33	27	22·02	·19
33	15	18·44	·18	33	28	22·50	·20
33	16	18·91	·18	33	29	22·92	·22
33	17	19·20	·21	33	30	23·35	·21

EXPERIMENT B. TABLE 8 (continued).

Growth of Female White Mice Receiving Vegetable Nucleic Acid and Thyroid, 1929-1932.

Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.	Number of animals.	Age in weeks.	Mean weight in gms.	Probable error of mean.
33	32	23·98	·28	23	90	28·04	·54
33	34	24·05	·30	23	92	27·59	·57
33	36	24·59	·32	23	94	27·41	·60
33	38	24·64	·31	20	96	27·42	·55
33	40	24·82	·31	20	98	26·92	·60
33	42	25·32	·32	18	100	26·83	·72
33	44	25·77	·34	17	102	26·32	·70
33	46	26·09	·34	17	104	25·85	·63
33	48	26·48	·33	16	106	25·62	·75
33	50	26·62	·35	16	108	25·19	·85
33	52	26·08	·36	14	110	25·21	·75
33	54	26·24	·36	14	112	24·68	·80
33	56	26·55	·38	13	114	24·85	·82
33	58	26·55	·41	13	116	25·15	·87
32	60	27·09	·40	12	118	25·04	·86
32	62	27·23	·42	12	120	25·00	·92
32	64	27·31	·38	11	122	25·00	·97
32	66	27·42	·38	9	124	24·89	·98
32	68	27·39	·45	8	126	25·25	1·08
32	70	27·53	·50	6	128	24·41	1·52
31	72	28·13	·50	6	130	24·58	1·36
30	74	28·15	·51	6	132	24·67	1·27
29	76	27·69	·55	5	134	24·50	1·53
29	78	27·21	·55	4	136	25·00	—
29	80	27·16	·56	2	138	28·75	—
29	82	27·38	·58	2	140	31·25	—
28	84	26·88	·59	2	142	30·75	—
26	86	28·02	·56	2	144	31·50	—
26	88	27·27	·52	2	146	32·00	—

EXPERIMENT A. TABLE 9.

Mortality Statistics.

At Age in Days.	Per cent. of Survivors. Male Animals. Control.	Male Animals. Experimental.	Female Animals. Control.	Female Animals. Experimental.
200	100	100	100	100
250	100	100	100	100
300	100	100	100	100
350	100	100	100	100
400	100	100	100	100
450	97·0	100	94·4	100
500	93·9	97·1	91·7	100
550	84·8	94·1	83·3	97·1
600	78·8	94·1	83·3	97·1
650	75·8	88·2	72·2	91·2
700	72·7	76·5	61·1	76·5
750	57·6	73·5	52·8	64·7
800	51·5	64·7	50·0	52·9
850	36·4	58·8	27·8	44·1
900	27·3	35·3	22·2	35·3

EXPERIMENT A. TABLE 9 (continued).

Mortality Statistics.

At Age in Days.	Per cent. of Survivors. Male Animals. Control.	Male Animals. Experimental.	Female Animals. Control.	Female Animals. Experimental.
950	12·1	23·5	16·7	11·8
1,000	3·0	8·8	8·3	8·8
1,050	3·0	5·9	5·6	0·0
1,100	0·0	0·0	0·0	—

Mean Duration of Life.

Male Animals	Control	772 days,	p.e. 20	days.
	Experimental	831 „	„ 17	„
	Difference	59 „	„ 26	„
Female Animals	Control	759 „	„ 20	„
	Experimental	813 „	„ 15	„
	Difference	54 „	„ 25	„

EXPERIMENT B. TABLE 10.

Mortality Statistics.

At Age in Days.	Per cent. of Survivors. Male Animals. Control.	Male Animals. Experimental.	Female Animals. Control.	Female Animals. Experimental.
200	100	100	100	100
250	97·2	100	100	100
300	97·2	100	100	100
350	97·2	100	100	100
400	97·2	97·1	97·2	100
450	94·4	97·1	91·7	96·9
500	91·7	94·3	91·7	96·9
550	83·3	88·6	91·7	87·5
600	72·2	85·7	91·7	78·1
650	69·4	82·9	83·3	68·7
700	66·7	68·6	77·8	59·4
750	66·7	62·9	61·1	50·0
800	52·8	51·4	55·6	40·6
850	44·4	45·7	47·2	34·4
900	27·8	42·9	36·1	18·7
950	16·7	22·9	19·4	12·5
1,000	13·9	8·6	5·6	6·2
1,050	13·9	5·7	2·8	0·0
1,100	2·8	0·0	0·0	—
1,150	2·8	—	—	—
1,200	0·0	—	—	—

Mean Duration of Life.

Male Animals	Control	775 days,	p.e. 23	days.
	Experimental	800 „	„ 20	„
	Difference	25 „	„ 30	„
Female Animals	Control	801 „	„ 19	„
	Experimental	750 „	„ 19	„
	Difference	51 „	„ 27	„

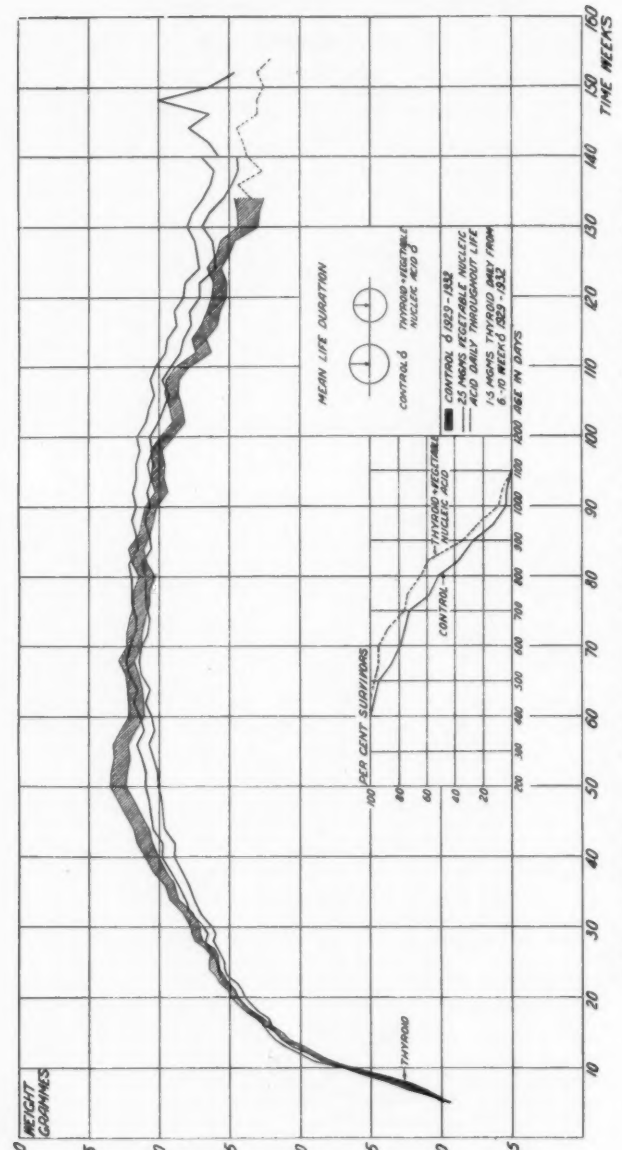

Fig. 1. Comparison of the growth and longevity of normal male mice and male mice in receipt of 25 mgs. of vegetable nucleic acid daily throughout life, and 1·5 mgs. ground thyroid tablets daily from 6–10 weeks of age.

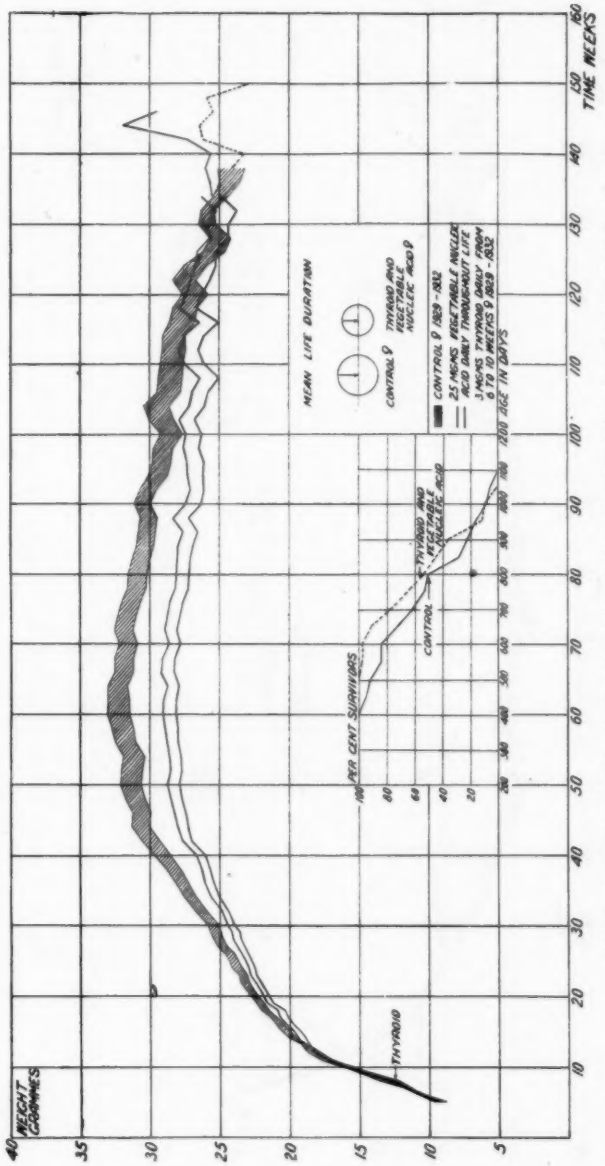
WEIGHT GRAMMES
TIME WEEKS
MEAN LIFE DURATION
PER CENT SURVIVORS
THYROID
CONTROL
THYROID AND VEGETABLE NUCLEIC ACID
CONTROL ♀ THYROID AND VEGETABLE NUCLEIC ACID ♀
CONTROL ♀ 1069 - 832
25 MGMS VEGETABLE NUCLEIC ACID DAILY THROUGHOUT LIFE
3 MGMS THYROID DAILY FROM 6 TO 10 WEEKS ♀ 1069 - 832
AGE IN DAYS

Fig. 2. Comparison of the growth and longevity of normal female mice and female mice in receipt of 25 mgs. of vegetable nucleic acid daily throughout life, and 3·0 mgs. ground thyroid tablets daily from 6–10 weeks of age.

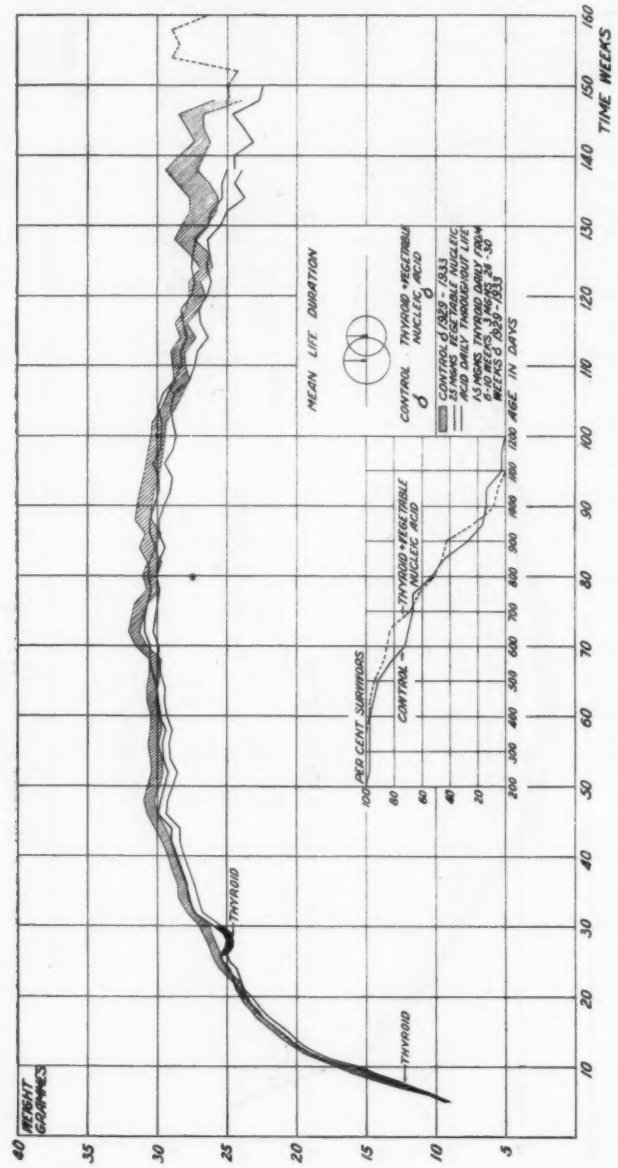

Fig. 3. Comparison of the growth and longevity of normal male mice and male mice in receipt of 25 mgs. of vegetable nucleic acid daily throughout life, and 1·5 mgs. ground thyroid tablets daily from 6–10 weeks of age, and 3·0 mgs. daily from 26–30 weeks of age.

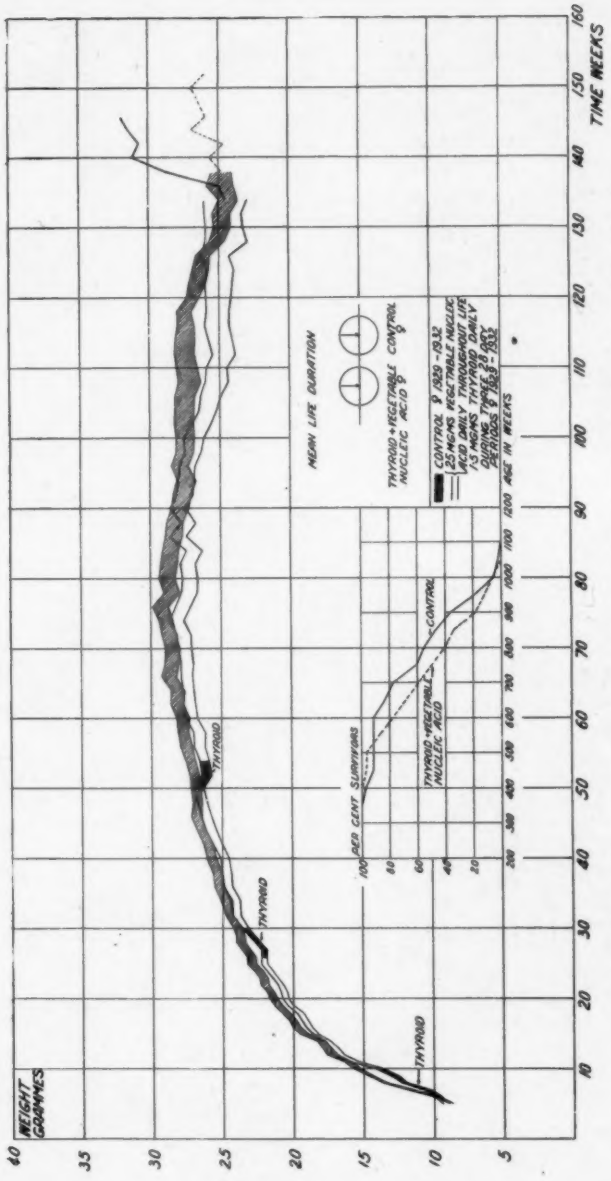

Fig. 4. Comparison of the growth and longevity of normal female mice and female mice in receipt of 25 mgs. of vegetable nucleic acid daily throughout life, and 1·5 mgs. ground thyroid tablets daily from 6–10, 26–30, and 50–54 weeks of age.